IN VITRO ACTIVITY OF FOSFOMYCIN-GENTAMICIN, FOSFOMYCIN-CEFTAZIDIME, FOSFOMYCIN-IMIPENEM AND CEFTAZIDIME-GENTAMICIN COMBINATIONS AGAINST CEFTAZIDIME-RESISTANT *PSEUDOMONAS AERUGINOSA*

Pornpimol Pruekprasert and Wanutsanun Tunyapanit

Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

Abstract. The effects of antimicrobial combinations against ceftazidime-resistant *Pseudomonas aeruginosa* strains isolated from hospitalized patients were investigated. Using the checkerboard titration method, combination of fosfomycin-gentamicin, fosfomycin-ceftazidime, fosfomycin-imipenem and ceftazidime-gentamicin was synergistic against 4, 11, 38 and 39% of 22, 18, 29 and 18 strains tested respectively and additive effect of the combinations against the strains tested was 41, 33, 14 and 44%, respectively. Antagonistic effects against the isolates were noted when fosfomycin was combined with gentamicin (27%), ceftazidime (22%) and imipenem (7%). No antagonistic effect was observed in the ceftazidime-gentamicin combination.

INTRODUCTION

Pseudomonas aeruginosa is a major opportunistic bacterial pathogen of nosocomial infections. The multiresistant strains play an important role in the colonization or infection of chronically hospitalized patients. Treatment is difficult and mortality significant. Beta-lactam antibiotics (ceftazidime), aminoglycosides (gentamicin, amikacin), fluoroquinolone (ciprofloxacin) and carbapenem (imipenem) have been mainstays for the treatment of *P. aeruginosa* infection. It is well known that the intensive use of antimicrobial agents inevitably leads to the appearance of organisms resistant to the drugs (Chen *et al*, 1995; Oie *et al*, 1999; Cavallo *et al*, 2000; Premru and Zupanc, 2002). Currently the treatment for *P. aeruginosa* infections is based on combinations of various antimicrobial agents because a single antimicrobial agent is often ineffective (Hilf *et al*, 1989; Korvick and Yu, 1991). The most widely documented synergy is to be found when beta-lactams are combined with aminoglycoside. The effectiveness of these combinations is limited due to the increasing resistance of *P. aeruginosa* to beta-lactams, and toxicity associated with aminoglycoside therapy (Hallander *et al*, 1982). For this reason, there is a continuous search for alternative combinations.

Fosfomycin, a bactericidal antibiotic, interferes with cell wall synthesis in both gram-positive and gram-negative bacteria by inhibiting the initial step, which involves phosphoenolypuruvate synthetase (Kahan *et al*, 1974). A number of studies have shown that fosfomycin can act synergistically with beta-lactams, which inhibit the last step of bacterial cell wall synthesis, and with aminoglycosides which inhibit bacterial protein synthesis (Olay *et al*, 1978; Takahashi and Kanno, 1984; Chin *et al*, 1986; Hayami *et al*, 1999; Okazaki *et al*, 2002). In this study, we evaluated the synergistic effects of fosfomycin in combination with ceftazidime, gentamicin and imipenem and the synergistic effects of ceftazidime in combination with gentamicin against ceftazidime-resistant *P. aeruginosa* strains isolated from hospitalized patients by using the checkerboard agar titration method.

MATERIALS AND METHODS

Bacteria

Fifty *P. aeruginosa* isolates used in this study were isolated from hospitalized patients at Songklanagarind Hospital, Prince of Songkla University, Songkhla, Thailand. Routine isolation,

Correspondence: Dr Pornpimol Pruekprasert, Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand.
Tel: 66 (0) 7445 1250-2

identification and susceptibility testing methods were performed at the Microbiology Laboratory of the Department of Pathology, Prince of Songkla University, Songkhla, Thailand between September 1997 and May 2003.

Antimicrobial agents

Standard laboratory powders of antimicrobial agents were supplied from Thai Meji Pharmaceutical (fosfomycin), Siam Pharmaceutical (ceftazidime), and Merck Sharp & Dohme, (imipenem). Standard powder of gentamicin was from Sigma Chemical and ceftazidime disks from Oxoid.

Media

Mueller-Hinton agar and Mueller-Hinton broth were used.

Susceptibility test, MIC determinations and FIC testing

The 50 *P. aeruginosa* isolates were shown to be ceftazidime-resistant strains by an agar disk diffusion susceptibility test (NCCLS, 1999).

The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined using a two-fold agar dilution method (NCCLS, 1999). The range of antimicrobial concentrations was 0.125-1,024 mg/l. Isolates with MIC ≤ 1,024 mg/l were used for the synergy studies.

The synergic effects of the antimicrobial combinations against selected isolates were evaluated using the checkerboard method. The ratio of antimicrobial agents was 1:1 and the concentration range was 0.125-1,024 mg/l. Fractional inhibitory concentrations (FICs) were calculated as the MIC of drug A and B in the combination divided by the MIC of drug A or B alone and the FIC index was obtained by adding the FICs. Synergism, addition, indifference and antagonism is defined as FIC index of ≤ 0.5, > 0.5 to 1, > 1 to 2, and > 2, respectively (Hayami *et al*, 1999).

RESULTS

The MIC, MIC_{50} and MIC_{90} values of the tested antimicrobial agents are shown in Table 1. On the basis of the NCCLS performance standards (NCCLS, 1999), all 50 isolates were resistant to ceftazidime, having MICs ranging from 64 to > 1,024 mg/l, with 23 isolates (46%) showing MICs greather than 1,024 mg/l. Almost all of the isolates were resistant to imipenem (98%) and gentamicin (100%). The MICs of fosfomycin ranged from 8 to > 1,024 mg/l. Thirteen (26%) and 21 (42%) isolates had MICs of gentamicin and fosfomycin over 1,024 mg/l. MIC, MIC_{50} and MIC_{90} for the combination of antimicrobial agents are shown in Table 2. The MIC_{50} levels of the combined antimicrobial agents were two to sixteen times lower than those of a single antimicrobial agent. The interactions between two combined antimicrobial agents, determined by checkerboard agar dilution, are shown in Table 3. The combination of fosfomycin with imipenem exerted a synergistic effect againts 11 isolates (38%), and 4 isolates (14%) showed an additive effect. Ceftazidime with gentamicin showed synergism with 7 isolates (39%) and additive effect with 8 isolates (44%). Synergistic and additive effects were noted less often in other combina-

Table 1
MIC value of fosfomycin, gentamicin, ceftazidime and imipenem against *P. aeruginosa*.

Antimicrobial agents	MIC (mg/l)		
	MIC range	MIC_{50}	MIC_{90}
Fosfomycin	8 - >1,024	512	>1,024
Gentamicin	8 - >1,024	1,024	>1,024
Ceftazidime	64 - >1,024	1,024	>1,024
Imipenem	4-64	64	64

Table 2
MIC values of fosfomycin-gentamicin, fosfomycin-ceftazidime, fosfomycin-imipenem and ceftazidime-gentamicin combinations against *P. aeruginosa*.

Antimicrobial agent combinations	MIC (mg/l)		
	MIC range	MIC_{50}	MIC_{90}
Fosfomycin-gentamicin	8-1,024	128	512
Fosfomycin-ceftazidime	8-1,024	256	1,024
Fosfomycin-imipenem	8-64	32	64
Ceftazidime-gentamicin	2-512	256	512

Table 3
Interactions between fosfomycin-gentamicin, fosfomycin-ceftazidime, fosfomycin-imipenem and ceftazidime-gentamicin as determined by checkerboard agar dilution against *P. aeruginosa.*

Antimicrobial agents (number of isolates)	Number of isolates (%)			
	Synergism (≤ 0.5)[a]	Additive (>0.5-1)[a]	Indifference (>1-2)[a]	Antagonism (>2)[a]
Fosfomycin-gentamicin (22)	1 (4.5%)	9 (40.9%)	6 (27.3%)	6 (27.3%)
Fosfomycin-ceftazidime (18)	2 (11.1%)	6 (33.3%)	6 (33.3%)	4 (22.2%)
Fosfomycin-imipenem (29)	11 (37.9%)	4 (13.8%)	12 (41.4%)	2 (6.9%)
Ceftazidime-gentamicin (18)	7 (38.9%)	8 (44.4%)	3 (16.7%)	0

[a]Fractional inhibitory concentration (FIC) index

tions. Antagonistic effects were noted when fosfomycin was combined with imipenem (7%), ceftazidime (22%) and gentamicin (27%). No antagonistic effect was noted in ceftazidime and gentamicin combination.

DISCUSSION

The incidence of multi-drug resistant *P. aeruginosa* strains isolated in hospitals continues to increase. This study was carried out in order to improve the efficacy of treatment. Judging from the MIC_{50} findings, combined antimicrobial agents showed higher activity than a single antimicrobial agent. Chin *et al* (1986) reported that the combination of fosfomycin with imipenem was synergistic against 45% of 49 *P. aeruginosa* strains tested comparable to our finding of 38%. Chin *et al* (1986) and Hayami *et al* (1999) reported that the synergistic activity of fosfomycin and ceftazidime combination against 62 and 26 *P. aeruginosa* strains tested was 31% and 27%, respectively slightly higher than our finding of 11%. The majority of the isolates used in this study was extremely resistant to the testing agents, according to the very high MIC_{50} and MIC_{90} findings. Unfortunately in the majority of isolates, the MICs of the combined antimicrobial agents were higher than the plasma concentrations of both drugs that can be achieved and much higher than the MIC breakpoint, on the basis of the NCCLS performance standards (NCCLS, 1999). Nevertheless, the results in this study suggest that the combination of fosfomycin with imipenem and ceftazidime with gentamicin might be alternatives for the treatment of serious infections due to *P. aeruginosa*. However, further studies of other antimicrobial combinations need to be done to combat infections due to increasingly drug resistant organisms.

ACKNOWLEDGEMENTS

The authors are grateful to the Microbiology Laboratory of the Department of Pathology, Prince of Songkla University for the kind donation of *P. aeruginosa* strains .This study was partly supported by the Thai Meji Pharmaceutical Co, Ltd.

REFERENCES

Cavallo JD, Fabre R, Leblanc F, Nicolas-Chanoine MH, Thabaut A, The GERPB. Antibiotic susceptibility and mechanisms of β-lactam resistance in 1,310 strains of *Pseudomonas aeruginosa*: a French multicentre study (1996). *J Antimicrob Chemother* 2000; 46: 133-6.

Chen HY, Yuan M, Ibrahim-Elmagboul IB, Livermore DM. National survey of susceptibility to antimicrobials amongst clinical isolates of *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1995; 35: 521-34.

Chin NX, Neu NM, Neu HC. Synergy of fosfomycin with beta-lactam antibiotics against Staphylococci and aerobic gram-negative bacilli. *Drugs Expt Clin Res* 1986; 12: 943-7.

Hallander HO, Dornbusch K, Gezelius L, Jacobson K, Karlsson I. Synergism between aminoglycosides

and cephalosporins with antipseudomonal activity: interaction index and killing curve method. *Antimicrob Agents Chemother* 1982; 22: 743-52.

Hayami H, Goto T, Kawahara M, Ohi Y. Activities of β-lactams, fluoroquinolones, amikacin and fosfomycin alone and in combination against *Pseudomonas aeruginosa* isolated from complicated urinary tract infections. *J Infect Chemother* 1999; 5: 130-8.

Hilf M, Yu VL, Sharp J, Zuravleff JJ, Korvick JA, Muder RR. Antibiotic therapy for *Pseudomonas aeruginosa* bacteremia: outcome correlations in a prospective study of 200 patients. *Am J Med* 1989; 87: 540-6.

Kahan FM, Kahan JS, Cassidy PJ, Kropp H. The mechanism of action of fosfomycin. *Ann NY Acad Sci* 1974; 235-364.

Korvick JA, Yu VL. Antimicrobial agent therapy for *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991; 35: 2167-72.

NCCLS. Performance standards for antimicrobial susceptibility testing. 9th ed. Informational supplement M100-S9. Wayne Pa: NCCLS, 1999.

Oie S, Sawa A, Kamiya A, Mizuno H. *In vitro* effects of a combination of antipseudomonal antibiotics against multi-drug resistant *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1999; 44: 689-91.

Okazaki M, Suzuki K, Asano N, *et al*. Effectiveness of fosfomycin combined with other antimicrobial agents against multidrug-resistant *Pseudomonas aeruginosa* isolates using the efficacy time index assay. *J Infect Chemother* 2002; 8: 37-42.

Olay TA, Rodriguez A, Oliver LE, Vincente MV, Oueredo MCR. Interaction of fosfomycin with other antimicrobial agents: *in vitro* and *vivo* studies. *J Antimicrob Chemother* 1978; 4: 569-76.

Premru MM, Zupanc TL. Epidemiological typing of imipenem-resistant *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2002; 20: 380-3.

Takahashi K, Kanno H. Synergistic activities of combination of betalactams, *fosfomycin*, and *tobramycin* against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1984; 26: 789.