The Journal of Antibiotics (2012) 65, 83–86

www.nature.com/ja

NOTE

Post-antibiotic effect of colistin, alone and in combination with amikacin, on *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients

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The Journal of Antibiotics (2012) **65,** 83–86; doi:10.1038/ja.2011.101; published online 30 November 2011

Keywords: antibiotic combinations; colistin; cystic fibrosis; post-antibiotic effect (PAE); *Pseudomonas aeruginosa*

Cystic fibrosis (CF) is a life-threatening, genetically inherited disease, especially common among people of Caucasian origin, and is characterized by recurrent lower respiratory tract infections. *Pseudomonas aeruginosa* is the predominant respiratory pathogen and is isolated from about 80% of the CF patients over their life times.[1] Treatment and prevention of *P. aeruginosa* infections in CF patients is a major problem.

As *P. aeruginosa* is a difficult organism to treat in most infections, the inadequacy and deficiency of effective antibiotics has led researchers to search for antimicrobial agents from natural sources. Among them, colistin, a polypeptide antibiotic, was first isolated in Japan from *Bacillus polymyxa* var. *colistinus* in 1947, and became available for clinical use in 1959. However, it was replaced in the 1970s by antibiotics considered less toxic.[2] Although it has been almost 50 years since its discovery and introduction into clinical use, colistin was never subjected to the drug development process. There are still inadequate data about its dosage recommendations.

However, not only the selection of the right antibiotic to treat these kinds of infections, but also using them in an optimal dosing interval affects the success of the treatment. As a pharmacodynamic parameter, the importance of post-antibiotic effect (PAE) is that antibiotics or antibiotic combinations that induce a long PAE may be administered with longer dosing intervals without loss of efficacy, thus letting the patients suffer less.[3,4]

As the PAE of colistin is largely unknown, the aim of our study was to investigate the PAE of this antibiotic, alone and in combination with amikacin, against *P. aeruginosa* strains isolated from CF patients.

EXPERIMENTAL PROCEDURE

Bacterial isolates

Seven strains of *P. aeruginosa* isolated from different CF patients (PA1–PA7) were obtained from sputum and throat secretion specimens submitted to the Clinical Microbiology Laboratories of Istanbul University, Istanbul Faculty of Medicine. All strains were identified by the API 20 NE System (bioMerieux Vitek, Marcy l'Etoile, France). *P. aeruginosa* ATCC 27853 was used as a quality-control strain.

Antibiotics

Colistin was obtained from Sigma Aldrich (St Louis, MO, USA) and amikacin was kindly provided from Eczacibasi Pharmaceuticals (Istanbul, Turkey). Stock solutions from dry powders were prepared at a concentration of 5120 mg l^{-1} and stored frozen at −80 °C. Frozen solutions of antibiotics were used within 6 months.

Media

Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) supplemented with divalent cations to a final concentration of 25 mg of Mg^{2+} and 50 mg of Ca^{2+} per liter (CSMHB) was used for minimum IC (MIC) determinations, microbroth checkerboard technique and for PAE experiments. Pour plates of Tryptic soy agar (Difco Laboratories) were used for colony counts.

Determination of MIC

Colistin and amikacin MICs were determined by the microbroth dilution technique as described by CLSI.[5,6] The MIC was defined as the lowest concentration of antibiotic giving complete inhibition of visible growth. Experiments were performed in triplicate.

Determination of fractional IC index

The effects of antibiotics in combination were assessed by using the microbroth checkerboard technique.[7,8]

Determination of the PAE

PAEs were determined by a standard viable counting method.[3] At time zero, 3 ml inoculum was added to tubes containing CSMHB, with or without test antibiotics. Organisms in the logarithmic phase of growth, producing a final concentration of inoculum in the test tubes of approximately 1×10^6 cfu ml^{-1}, were exposed to concentrations of colistin and amikacin equal to 1× or 20× MIC, alone and in combinations. After incubation for 1 h in a 37 °C-calibrated

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Received 27 October 2010; revised 4 October 2011; accepted 7 October 2011; published online 30 November 2011

shaking water bath, antibiotics were removed by washing the contents of the tubes twice with CSMHB. After centrifugation for 10 min at 5000 r.p.m., pellets were resuspended in prewarmed broth; controls were handled similarly. Bacterial counts of the tube contents were determined at time zero, immediately before and after centrifugation, and each hour after centrifugation for 8 h by a pour-plate technique, using appropriate dilutions. The PAE was defined as $PAE=T-C$, where T is the time (in hours) required for the count in the test culture to increase one $\log_{10}$ above the count observed immediately after centrifugation, and C is the corresponding time for the controls. Experiments were performed in triplicate.

Statistical analysis

Statistical analysis for the comparison of PAEs was performed using the non-parametric analysis of variance and Bonferroni correction for *post-hoc* comparisons. All data were reported as mean ± s.e. Any value of P below 0.05 was considered as statistically significant.

RESULTS

The MICs of colistin and amikacin ranging from 0.5 to 1 mg l^{-1} are shown in Table 1. In this study, two clinical strains demonstrated synergistic interactions and no antagonism was observed with any combination (Table 1). Both of the antibiotics showed increased PAE values in a concentration-dependent manner. PAE values of colistin and amikacin, alone and in combination, against seven clinical *P. aeruginosa* strains are summarized in Table 2. When the antibiotics were used in combination at a concentration of 20× of the MIC values, the PAEs were prolonged to 3.79 ± 0.19 h (Figure 1).

For the control strain *P. aeruginosa* ATCC 27853, colistin at a concentration equal to and 20× of MIC values, exhibited 1.5 and 2.2 h, and amikacin 1.5 and 2.5 h of PAEs, respectively; and when antibiotics were used in combination at 20× MIC, the PAE was prolonged to 3.9 h.

DISCUSSION

PAE is a well-established pharmacodynamic parameter that describes the duration of antimicrobial effect after the active concentration of antibiotics has been removed from the culture medium.[3] Besides quantitative pharmacodynamic parameters, such as AUC_{0-24}:MIC, Cmax:MIC and T > MIC, which have been proposed as likely predictors of clinical and microbiological success, PAE provides potential influence on antimicrobial dosing regimens in clinical practice, where agents inducing a long PAE may be administered with longer dosing intervals than currently employed, without loss of efficacy.[9]

Our experiments showed that durations of the PAE of amikacin were significantly increased in a concentration-dependent manner. When the concentration was increased to 20× MIC, the increase in duration of PAEs were almost 2.5 times longer than when the agent was applied at concentrations equal to 1× MIC ($P<0.001$; Figure 1). In addition to concentration-dependent bactericidal activities of aminoglycosides, prolonged PAE is an important parameter for once-daily dosing treatments with longer dosing intervals. In addition to their favorable antimicrobial activity against *P. aeruginosa* strains, aminoglycosides can suppress nonsense mutations located in defective *CFTR* genes and thus permit translation to continue to the natural termination codon in CF patients.[10] Among them, amikacin suppresses the premature stop codon mutation more effectively than gentamicin, when administered at clinically relevant doses.[11] This finding was also one of the reasons why we have chosen this antibiotic as a combination partner.

Although colistin was developed in the 1960 s, it has been recently met with renewed interest because of its significant activity against multidrug-resistant strains of Gram-negative pathogens, especially strains of *P. aeruginosa* and *Acinetobacter baumannii*. Because of inadequate data, its pharmacodynamic parameters have not been defined sufficiently.[12] For that reason, our main interest was to

Table 1 ***In-vitro*** **activities of colistin and amikacin, alone and in combination, against clinical** ***P. aeruginosa*** **strains (PA1-7) and ATCC 27853 control strain**

	MIC (mg l^{-1})		FIC index[a]
Isolate	*Colistin*	*Amikacin*	*Colistin+amikacin*
PA1	0.5	0.5	1
PA2	1	1	1
PA3	1	0.5	1
PA4	1	1	1
PA5	1	1	0.5
PA6	1	1	1
PA7	1	1	0.5
ATCC 27853	1	1	1

Abbreviations: FIC, fractional IC; MIC, minimum IC.
[a]FIC ⩽0.5 synergistic effect.

Table 2 PAE values (h) of antibiotics, alone and in combination, against seven clinical ***P. aeruginosa*** **strains isolated from CF patients**

Strains	*C*	*20C*	*A*	*20A*	*A+C*	*A+20C*	*20A+C*	*20A+20C*
PA1	1.5	2.85	1.4	3.75	1.8	4.15	4.2	4.8
PA2	1.2	1.9	1.2	2.5	1.3	2.6	2.9	3.8
PA3	0.95	1.7	1	2.2	1.4	2.4	2.65	3.3
PA4	1.3	2	0.7	2.4	1.75	2.3	2.6	3.5
PA5	1	2.4	0.6	3.1	1.6	2.5	2.6	4
PA6	1	2	0.9	2	1.3	2.3	2.7	3.4
PA7	1	2	1,2	2.2	1.3	2.5	2.8	3.7

Abbreviations: A, amikacin; C, colistin; CF, cystic fibrosis; MIC, minimum IC; PAE, post-antibiotic effect.
C, colistin equal to the MIC; 20C, C equal to the 20× MIC; A, amikacin equal to the MIC; 20A, A equal to the 20× MIC.

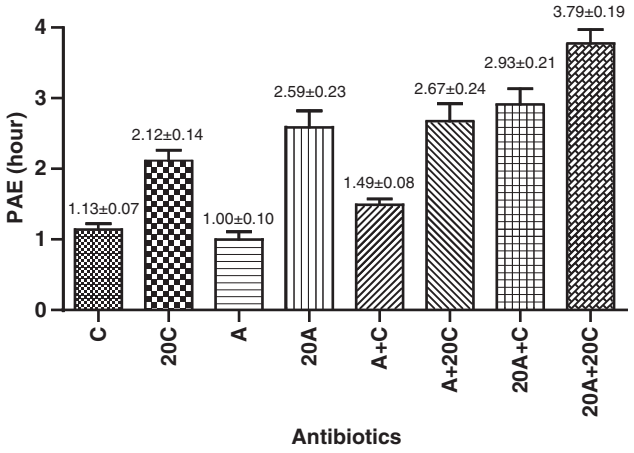

Figure 1 Post-antibiotic effect (PAE) values of antibiotics, alone and in combination, on seven clinical strains of *P. aeruginosa* isolated from cystic fibrosis (CF) patients. C: colistin equal to the minimum IC (MIC); 20C: C equal to the 20× MIC; A: amikacin equal to the MIC; 20A: A equal to the 20× MIC.

evaluate the PAE of colistin, alone and in combination with amikacin, against *P. aeruginosa* strains isolated from CF patients. According to our results, colistin produced a significant PAE of 2.12 ± 0.14 h against the strains at 20× MIC concentrations, which is in agreement with results obtained by other investigators.[12] We have seen almost the same pattern as we did with amikacin. When the applied concentration of colistin was increased from 1× MIC to 20× MIC, the duration of PAE was extended two times longer ($P<0.001$; Figure 1). Like aminoglycosides, colistin possesses a concentration-dependent bactericidal activity and prolonged PAE. From these points of view, colistin could also be considered as an option for a once-daily dosing treatment for patients infected with *P. aeruginosa*. However, to establish an appropriate therapy protocol for once-daily dosing, additional clinical studies are required.

As colistin is a concentration-dependent antibiotic against Gram-negative bacteria, its bactericidal activity depends on the peak tissue concentrations reached in infected lung parenchyma.[13] Lung tissue penetration of colistin following i.v. infusion is unknown, and pharmacokinetic studies have been reported recently. However, in a recent experimental study which was performed in anesthetized and ventilated piglets with inoculation pneumonia caused by *P. aeruginosa*, it was shown that high-colistin lung tissue deposition and antibacterial efficiency following nebulization was detected, contrasting with the absence of any detectable deposition following i.v. administration.[14] It is well known, nebulization of antibiotics offers the possibility of generating high-drug concentrations at the site of infection. There are several studies that have demonstrated clinical use of nebulized colistin suggests good efficiency.[15] Additionally, it was also reported that a single nebulization of colistin was associated with long-lasting high-sputum concentrations in patients with CF.[16] It was reported that serum peak concentration of colistin is $18\,\mathrm{mg\,l^{-1}}$.[17] As we obtained low MIC values of colistin, we chose 20× MIC, as well as 1× MIC of colistin in PAE experiments.

On the other hand, i.v. aminoglycosides easily penetrate in lung parenchyma and bronchial secretions. However, lung tissue concentrations remain small, because plasma levels are kept low to avoid toxicity. Aerosol administration offers the theoretical advantage of high concentrations of antibiotics at the site of infection together with a low systemic absorption, resulting in reduced renal toxicity. Several studies have showed the lung tissue concentrations after the administration of i.v. aminoglycosides; Santré *et al.* reported[18] a peak concentration of amikacin of $14.9\,\mathrm{mg\,l^{-1}}$ in bronchial secretions. In another study, a similar tissue peak concentration ($13\,\mathrm{mg\,l^{-1}}$) was obtained for amikacin.[19]

Besides applying the antibiotics in appropriate dosing regimens, another way of getting over problems of resistance during the treatment of chronic *P. aeruginosa* infections in CF patients is the use of antibiotics in combination. *In-vitro* and *in-vivo* trials have shown that, to produce rapid enhancement of bactericidal activity and to help prevent or delay the emergence of resistance, combination therapy with two antipseudomonal antibiotics is superior to monotherapy, and results in a more prolonged clinical remission in CF patients who are infected with *P. aeruginosa*.[20] There are few experimental and clinical studies in the literature regarding combinations of colistin with other antibiotics, such as beta-lactams, rifampin, amikacin, trimethoprim-sulfomethoxazole and ciprofloxacin, against multidrug-resistant Gram-negative bacteria.[13,21–24] In a recent study, we have shown the existence of *in-vitro* synergy between colistin methanesulfonate and amikacin in 12% of 50 *P. aeruginosa* strains isolated from CF patients.[25] In the present study, synergistic interactions were seen in two of seven clinical strains. No antagonism was observed.

The PAE with certain antibiotic combinations against *P. aeruginosa* have been published,[26–29] but colistin combinations have not been reported. The present study was aimed to determine the PAE of colistin and amikacin in combination at low and higher concentrations. Accordingly, although the use of antibiotics in combination at MIC concentrations increased the duration of PAE when tested alone, this increase has not been found statistically meaningful ($P>0.05$). On the other hand, PAE values that have been found by using antibiotics alone at high concentrations like 20× MIC, were significantly higher than the values they created in combination at low concentrations like 1× MIC ($P<0.01$ for colistin and $P<0.001$ for amikacin). However, when they were used in combination, increasing one of the antibiotics' concentrations to 20× MIC significantly increased the duration of PAE than when used alone or in combination at 1× MIC ($P<0.001$). When concentrations of both antibiotics in the combination were increased to 20× of the MIC values, the duration of PAE extended to the highest observed value (3.79 ± 0.19 h, $P<0.001$). These results revealed the significant existence of a concentration-dependent prolonged PAE of colistin and amikacin not only when they were used alone, but also in combination.

These results indicate that colistin and amikacin might be administered in longer time intervals than those already applied. It remains to be demonstrated in a clinical setting that a modified dosage scheme based on prolonged PAE would preserve colistin activity and at the same time, improve its safety profile.

In conclusion, taken together, the results of this study may have a useful role in selecting the appropriate timing of doses during therapy with colistin and amikacin, alone or in combinations, to treat CF patients with *P. aeruginosa* infections.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Research Fund of The University of Istanbul. Project number: T–816/27122005. We thank Zayre Erturan, for supplying the *P. aeruginosa* isolates.

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