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Postantibiotic effects of eleven antimicrobials on five bacteria[1]

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ABSTRACT

AIM: To investigate the postantibiotic effects (PAE) of different classes of antimicrobials against five different types of bacteria. **METHODS**: Minimal inhibitory concentrations (MIC) were determined by twofold macrodilution in broth. The antimicrobial agents were eliminated by washing method after the bacteria were exposed to antimicrobials for 1 h or 2 h. Growth curves were followed by viable counts, and then the PAE were calculated. **RESULTS**: Macrolides induced PAE of 3.10 h to 4.15 h on *S aureus*, and 1.85 h to 3.3 h against *S pneumoniae*, which were longer than PAE induced by other tested antimicrobials ($P < 0.01$). Macrolides induced PAE of 1 h to 4 h against *H influenzae*, with azithromycin producing the longest PAE of 4 h. Ciprofloxacin and amikacin induced PAE of 1.38 h to 2.00 h on *E coli* and *K pneumoniae*, which were longer than that of β-lactams, piperacillin, cefazolin, or cefotaxime, with PAE of 0.1 h to 0.5 h ($P<0.01$). **CONCLUSION**: Different classes of antimicrobials induce different periods of PAE. As an important pharmacodynamic parameter, PAE provide reference data for the determination of the optimal dosing regimen and reasonable use of antimicrobials.

INTRODUCTION

Suppression of bacterial growth that persists after short exposure of organisms to antimicrobials has been observed since the early 1940s, but it was not until the last 20 years that researchers extended early observation to other common antimicrobials and microorganisms. The term "postantibiotic effect" (PAE) is used to describe the recovery period or persistent suppression of bacterial growth after short antimicrobial exposure. Now PAE is thought to be an important pharmacodynamic parameter and has been increasingly applied for the design of antimicrobial dosing regimens. The aim of this study was to investigate the PAE of 4 classes of antimicrobials (macrolides, β-lactams, fluoroquinolones, and aminoglycosides) against clinically common pathogens, *S aureus*, *S pneumoniae*, *H influenzae*, *E coli*, and *K pneumoniae*.

MATERIALS AND METHODS

Antimicrobial agents Antimicrobials were obtained as reference powders from the indicated companies or institute: penicillin, Shanghai Pioneer Pharmaceutical Cooperation; ampicillin, Shanghai Fourth Pharmaceutical Ltd; cefuroxime, Glaxo Wellcome Pharmaceuticals Ltd; clarithromycin, Abbott Laboratories; azithromycin, Pfizer Pharmaceutical Ltd; roxithromycin, Hoechst Marion Roussel; ciprofloxacin, Shanghai Sunve Pharmaceutical Co Ltd; piperacillin, cefazolin, erythromycin, and amikacin, National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Bacteria The bacterial strains used in this study were as follows: *Staphylococcus aureus* ATCC25923, *Streptococcus pneumoniae* CDC81-7801, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCTC9633. *Haemophilus influenzae* 94046 was a clinical strain obtained from Huashan Hospital, Fudan University.

Culture media ISO-SENSITEST broth (Unipath Ltd) was used for the culture of *S aureus*, *E coli*, and *K pneumoniae*, Heart Brain Infusion (HBI, Difco Laboratories) for *S pneumoniae*, HBI supplemented with 5 % Fildes enrichment for *H influenzae*. Mueller-

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Hinton agar (Oxoid Ltd) was used for *S aureus*, *E coli*, and *K pneumoniae*, MH Agar supplemented with 5 % sheep blood for *S pneumoniae*, chocolate plate for *H influenzae*.

Determination of minimal inhibitory concentrations (MIC) MIC of antimicrobials against bacteria were determined by twofold broth dilution method as recommended by NCCLS.

Determination of PAE The PAE were determined with the method of Craig NA and Gudmundsson S[11], as follows: One or two colonies of bacterium was incubated into the test broth at 35 ℃ the night before, and the overnight culture was diluted 100- or 1000-fold with test broth and incubated for further 2 – 3 h to obtain the bacterium in logarithmic phase of growth. The optical density of inoculum was tested with spectrophotometer. Inoculum of 5×10^7 to 1×10^8 (colony-forming units) CFU/mL 1 mL was then added to test tube and control tube, containing 8.9 mL and 9.0 mL test broth previously warmed, respectively. Antimicrobial solution 100 μL previously prepared was added to the test tube to reach the desired test concentration. The exposure concentrations of antimicrobials were 4 × MIC for *S aureus* and *S pneumoniae*, 8 × MIC for *H influenzae*, and 2 × MIC for *E coli* and *K pneumoniae*. The test tube and control tube were incubated in incubator with continuous shaking, *H influenzae* and *S pneumoniae* was incubated in 5 % CO_2. The antimicrobial exposure time was 2 h for *S aureus*, *S pneumoniae*, and *H influenzae*, 1 h for *E coli* and *K pneumoniae*. The antimicrobial was removed by washing. The supernatant was decanted after centrifugation and the organisms were resuspended in prewarmed fresh medium. The same procedure was performed 1 or 2 additional times.

Counts of CFU/mL were performed on all cultures with viable counts at the begining time, before and after washing, and every 1 h thereafter. After a serial 10-fold dilution of withdrawn inoculum, an 100-μL sample was plated on agar plate. Plates were read after incubated for 24 h.

The counts of CFU/mL were graphed as in Fig 1. The duration of PAE was calculated according to the following formula: PAE = $T - C$, where T is the time required for the viable count of the test culture to increase by 1 logarithmic unit above the counts observed immediately after washing and C is the corresponding time for the control. The results were based on the means from 3 experiments.

Statistics PAE were expressed as $\bar{x} \pm s$ and assessed by analysis of variance.

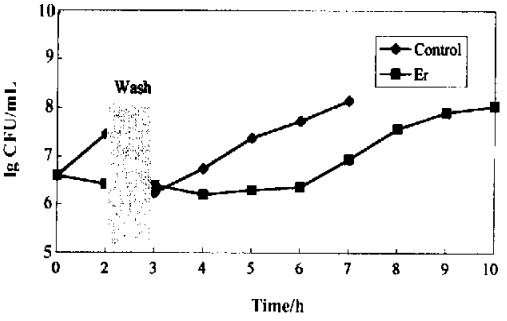

Fig 1. The PAE of erythromycin against *S pneumoniae* exposured for 2 h to a concentration of 0.125 mg/L (4 × MIC).

RESULTS

The MIC and PAE for the different strains are listed in Tab 1.

Macrolides (erythromycin, azithromycin, clarithromycin, and roxithromycin) induced PAE generally of 3.10 h to 4.15 h against *S aureus*, and 1.85 h to 3.3 h on *S pneumoniae*. β-Lactams, penicillin and cefazolin, produced PAE between 0.90 h and 1.48 h against *S aureus*, while 1.21 h and 1.28 h for ciprofloxacin against *S aureus* and *S pneumoniae*, respectively. Macrolides induced longer PAE on *S aureus* and *S pneumoniae* than did penicillin, cefazolin, and ciprofloxacin, and the difference was significant ($P < 0.01$).

Macrolides induced PAE generally between 1 h and 4 h against *H influenzae* and azithromycin induced longer PAE of 4 h than that of erythromycin, clarithromycin, and roxithromycin ($P < 0.01$). Cefuroxime produced no PAE, but ampicillin induced relatively long PAE on *H influenzae*.

Ciprofloxacin and amikacin induced longer PAE of 1.61 h and 1.70 h on *E coli* than did β-lactams, piperacillin and cefazolin ($P < 0.01$). Ciprofloxacin and amikacin induced PAE of 1.38 h and 2.00 h on *K pneumoniae*, which were longer than that of β-lactams, piperacillin and cefotaxime ($P < 0.01$).

DISCUSSION

The design of antimicrobial dosing regimens is mainly based on the susceptibility of pathogen and pharmacokinetic parameters such as drug concentration in serum and tissue and elimination half-life. In conjunction

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Tab 1. Postantibiotic effects of antimicrobial agents on five bacteria. $n = 3$ experiments. $x \pm s$. [c]$P < 0.01$ *vs* penicillin. [f]$P < 0.01$ *vs* cefazolin. [i]$P < 0.01$ *vs* ciprofloxacin. [l]$P < 0.01$ *vs* azithromycin. [p]$P < 0.01$ *vs* piperacillin. [r]$P < 0.01$ *vs* cefotaxime.

	MIC/ mg·L^{-1}	Exposure concentration /mg·L^{-1}	Exposure time/h	PAE/h
S aureus ATCC25923				
Penicillin	0.03	0.125	2	1.5 ± 0.4
Cefazolin	0.125	0.50	2	0.90 ± 0.28
Erythromycin	0.25	1.0	2	3.10 ± 0.14[cfi]
Azithromycin	1.0	4.0	2	3.1 ± 0.5[cfi]
Clarithromycin	0.25	1.0	2	4.15 ± 0.28[cfi]
Roxithromycin	1.0	4.0	2	3.30 ± 0.26[cfi]
Ciprofloxacin	1.0	4.0	2	1.21 ± 0.06
S pneumoniae CDC78-8101				
Penicillin	0.25	1.0	2	1.40 ± 0.20
Cefazolin	0.5	2.0	2	0.95 ± 0.25
Erythromycin	0.03	0.125	2	3.3 ± 0.3[cfi]
Azithromycin	0.125	0.5	2	1.85 ± 0.05[cfi]
Clarithromycin	0.015	0.06	2	2.00 ± 0.30[cfi]
Roxithromycin	0.06	0.25	2	2.65 ± 0.05[cfi]
Ciprofloxacin	0.5	2.0	2	1.28 ± 0.18
H influenzae 94046				
Ampicillin	0.25	2	2	2.37 ± 0.21
Cefuroxime	1.0	8	2	−0.27 ± 0.06
Erythromycin	2.0	16	2	1.43 ± 0.15[l]
Azithromycin	1.0	8	2	4.00 ± 0.26
Clarithromycin	4.0	32	2	1.77 ± 0.25[l]
Roxithromycin	8.0	64	2	1.03 ± 0.21[l]
E coli ATCC25922				
Piperacillin	2	4	1	0.50 ± 0.14
Cefazolin	2	4	1	0.10 ± 0.20
Ciprofloxacin	0.015	0.03	1	1.61 ± 0.18[fo]
Amikacin	2	4	1	1.70 ± 0.42[fo]
K pneumoniae NCTC9633				
Piperacillin	4	8	1	0.07 ± 0.20
Cefotaxime	0.03	0.06	1	0.15 ± 0.23
Ciprofloxacin	0.06	0.125	1	2.00 ± 0.18[or]
Amikacin	1.0	2.0	1	1.38 ± 0.23[or]

with other pharmacodynamic parameters, PAE is, now, being applied increasingly to allow antimicrobial dosing regimens to be developed on a more scientific basis. Different antimicrobials induce varied duration of PAE against different types of bacteria. More prolonged intermittent dosing regimens would apply primarily to antimicrobials that exhibit a prolonged PAE. On the other hand, more continuous dosing would be necessary for antimicrobials that exhibit a shorter PAE or lack a PAE[1,5].

This study showed that macrolides, erythromycin, azithromycin, clarithromycin, and roxithromycin, induced pronounced PAE against common respiratory tract pathogens (*S pneumoniae*, *H influenzae*, and *S aureus*) and the PAE were significantly longer than that of other tested antimicrobials. Meanwhile the elimination half-lives of new macrolides are longer than that of erythromycin. The above data indicate that longer dosing intervals for new macrolides can be allowed. Once- to twice-daily dosing regimens are, now, applied in clinical practice and give high clinical efficacy rates. The results of this study corroborate with the findings of

Odenholt-Tornqvist[2]. They found that roxithromycin, clarithromycin, and azithromycin induced long PAE and PA SME (postantibiotic sub-MIC effects) on respiratory tract pathogens (*S pyogenes*, *S pneumoniae*, and *H influenzae*). The PAE were 2.9 – 8.8 h after pathogens were exposed to 2 × MIC or 10 × MIC of macrolides for 2 h.

β-Lactam antibiotics, penicillin and cefazolin, induced a short PAE of 0.9 to 1.5 h on gram-positive bacteria (*S aureus* and *S pneumoniae*), meanwhile piperacillin, cefazolin, and cefotaxime induced almost no PAE on gram-negative bacteria (*E coli* and *K pneumoniae*). Because β-lactams induce short or no PAE, the bactericidal activity and clinical efficacy depends mainly on duration of time that antimicrobial concentration exceeds the MIC, called time-dependent bactericidal activity, and has little relationship to the magnitude of drug concentration[3]. β-Lactams should be administered in a short interval.

This study showed that aminoglycoside, amikacin, induced a long PAE on gram-negative bacilli (*E coli* and *K pneumoniae*); fluoroquinolone, ciprofloxacin, induced long PAE against both gram-positive and gram-negative bacteria. The pharmacodynamic information that aminoglycosides and fluoroquinolones display concentration-dependent bactericidal activity and a long PAE established the foundation for infrequent, intermittent dosing regimen[3,4]. High-dose, extended-interval aminoglycoside therapy provides the opportunity to maximize the peak concentration/MIC ratio and the resultant bactericidal activity. Once-daily aminoglycoside dosing regimen optimizes potential for clinical cure and minimizes toxicity, and it may help to prevent the development of resistance[4]. Once-daily dosing regimen has also been implemented in clinical practice for some fluoroquinolones such as fleroxacin with a long PAE and long elimination half-life.

The mechanisms by which antimicrobials induce the PAE have not been clearly understood, but it is suspected that more than one mechanism exists. The two most commonly proposed mechanisms are drug-induced non-lethal damage and persistence of antimicrobial at the bacterial binding site[5]. β-Lactams bind covalently to multiple penicillin-binding proteins (PBP), some of which are enzymes involved in cell wall synthesis. PAE represents the time required by the organism to synthesize new PBP (new enzymes)[1]. Aminoglycosides probably cause irreversible binding of sub-lethal amounts of drug to the ribosome leading to disruption of protein synthesis. The PAE may represent the time needed for resynthesis of ribosomal protein[1,5]. In a recent study by Champney and Tober[6], the molecular mechanisms of PAE induced by erythromycin and clarithromycin on *S aureus* were investigated. They found that erythromycin and clarithromycin treatment markedly reduced the number of 50S ribosomal subunits and 90 min was required for resynthesis to give the control level. Protein synthesis rates were diminished for 3 – 4 h after the removal of macrolides. The PAE reflect the time required for the synthesis of new 50S subunits and the slow loss of the antibiotics from ribosomes in inhibited cells.

CONCLUSION

Different classes of antimicrobials induce different period of PAE. As an important pharmacodynamic parameter, PAE provide theoretical rationale for the determination of optical dosing schedule of antimicrobials.

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