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Impact of pH and Cationic Supplementation on In Vitro Postantibiotic Effect

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Most studies on pharmacodynamic variables in vitro, including the postantibiotic effect (PAE), are performed at pH 7.4 in noncationic-supplemented media, a situation which may differ significantly from the true microenvironment in most infected foci. We studied the impact of five different pH levels (pH 5, 6, 7, 7.4, and 8) on the duration of the PAE, the MIC, and bactericidal activity. Acid pH was found to have in general a deleterious effect on the activity of aminoglycosides and ciprofloxacin against *Escherichia coli* and *Pseudomonas aeruginosa*, with the MIC being higher, the bactericidal rate being lower, and the PAE being shorter at pH 5 (and to a lesser extent at pH 6) than at more alkaline pH levels. Similar results were observed for imipenem against *P. aeruginosa*. The PAEs induced by ampicillin against *E. coli* and dicloxacillin against *Staphylococcus aureus* were not predictably dependent on the pH, whereas the PAEs induced by ciprofloxacin against *S. aureus* were longest at either end of the pH spectrum. The bactericidal activity of these agents was, however, pH dependent, being slower at acid pHs. The addition of 50 mg of Ca^{2+} and 20 mg of Mg^{2+} per liter of liquid medium at pH 7.4 did not affect the duration of the PAE. Since the pH in abscess cavities may be close to 5, these observations may be of importance for employment of the agents studied in closed or poorly drained infections.

Several factors may affect the interaction of microorganisms and antibacterial agents at infected tissue sites, and the resulting antibacterial activity may differ markedly from what is observed in the controlled and standardized in vitro situation. Previous studies have demonstrated that low pH, high concentration of divalent cations, low redox potential, high protein content, microbial β-lactamase in pus, less activity of β-lactam drugs on poorly dividing organisms in pus, inhibition of bacterial metabolism by serum- and tissue-derived proteins, and the high bacterial inoculum in untreated abscesses may all adversely influence antibacterial activity (2, 3, 18, 26). The pharmacodynamic variables examined in these studies have been almost exclusively the MIC and MBC and to a lesser extent the bactericidal rate (4, 9, 19, 25, 29), but studies on other potentially important variables, e.g., the postantibiotic effect (PAE), have been limited (6).

The PAE, a term used to describe a suppression of bacterial growth that persists after a limited exposure of organisms to antimicrobial agents, has been shown to be a characteristic of virtually all antimicrobial agents tested so far in vitro and in vivo (6). The presence and duration of the PAE, however, differ significantly for various microorganism-antimicrobial agent combinations. PAE's importance as a pharmacodynamic parameter is primarily related to its potential influence on antimicrobial dosing regimens in clinical practice.

Most studies on antimicrobial activity in vitro are performed in commercially available liquid media at a pH that resembles that of human serum (pH 7.4). The pH of most infected foci, however, is probably much lower than that of serum (18). Limited data on the pH of abscess fluid supernatant have, for example, indicated values ranging from 5.5 to 6.8 (2, 3), and metabolic acidosis is a well-known complication of septic shock (11). The pH of infected urine is generally 5 to 6, although urine infected with urea-splitting organisms may reach a value of 8.0 or higher (21).

The mean concentration of calcium in abscess fluid has been found to be slightly lower (~60 mg/liter; range, 30 to 90 mg/liter) and magnesium concentrations slightly higher (~40 mg/liter; range, 30 to 50 mg/liter) than those ordinarily found in serum (2, 3). These concentrations, however, are higher than in commercially available liquid media usually employed for in vitro antibacterial tests.

We, therefore, examined the impact of five different pH levels from 5.0 to 8.0 on the duration of the PAE for three common pathogenic organisms after exposure to several antimicrobial agents in Ca^{2+}-Mg^{2+}-supplemented medium. The PAE was also determined in nonsupplemented medium at pH 7.4. Furthermore, the bactericidal rate during 1 h of exposure was determined as part of each experiment.

(This study was presented in part at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Tex., 18 to 20 September 1989 [10]).

MATERIALS AND METHODS

Organisms. The study organisms were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and B5756 (a clinical strain), and *Pseudomonas aeruginosa* ATCC 27853 and B 408 (a clinical strain). The clinical strains were obtained from the Clinical Microbiology Laboratory, Borgarspitalinn, Reykjavik, Iceland.

Antimicrobial agents. Dicloxacillin (Bristol Sermoneta, Latina, Italy), gentamicin (Roussel Laboratories Ltd., Uxbridge, United Kingdom), and ciprofloxacin (Bayer AG, Leverkusen, Germany) were used against *S. aureus*; ampicillin (Astra Läkemedel, Södertälje, Sweden), gentamicin, and ciprofloxacin were used against *E. coli*; and imipenem

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(Merck Sharp & Dohme International, Rahway, N.J.), tobramycin (Eli Lilly & Co. Ltd., Indianapolis, Ind.), and ciprofloxacin were used against *P. aeruginosa*. The antibiotics were diluted into solutions as recommended by the manufacturers to the desired concentration for each experiment. MICs were determined at each individual pH level by standard methods in microtiter plates (15).

Growth medium. Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich.) was used as a culture medium. The pH of the broth was adjusted to 5.0, 6.0, 7.0, 7.4, and 8.0 before sterilization by titration with 1.2 N HCl or NaOH as required. The pH was measured by a PHM84 Research pH meter (Radiometer, Copenhagen, Denmark). The stability of the pH at each level was checked after sterilization and before each experiment. In most instances, the pH was measured as well at the time of the last sampling when the tubes had turned cloudy (3 to 4.5 h for unexposed controls and 6 to 8 h for exposed cultures [see below]). Furthermore, in three or four experiments for each organism, the culture tubes were incubated for an additional 16 to 20 h and the pH was measured again approximately 24 h after initiation of the experiment.

The broth was supplemented by 50 mg of Ca^{2+} as calcium chloride and 20 mg of Mg^{2+} as magnesium sulfate per 1,000 ml (22). Since autoclaving may affect the Ca^{2+} and Mg^{2+} concentrations in MHB (14), the cations were added to the broth after sterilization. Each experiment was conducted with nonsupplemented MHB at pH 7.4 to examine the effect of divalent cation supplementation. The desired concentrations of Ca^{2+} and Mg^{2+} were verified by measuring the concentrations in the broth by standard methods.

Inoculum and drug exposure. Before each experiment, four or five colonies of the test organism were transferred to tubes containing 7 to 10 ml of MHB at each individual pH level and grown overnight (8 to 10 h) at 35.5°C. The cultures were then adjusted by a 0.5 McFarland standard to an inoculum of $\sim 10^7$ CFU/ml in the logarithmic phase of growth.

At each individual pH, the organisms were exposed to the antimicrobial agents for 1 h in 5 ml of broth at 35.5°C in heat blocks (Thermolyne Dri Bath; Thermolyne Corporation, Dubuque, Iowa) mounted on a suspension mixer (Model 802, Luckham, England). The following concentrations were employed: 4 to 8× the MIC of dicloxacillin, 1 to 2× the MIC of gentamicin, and 1 to 4× the MIC of ciprofloxacin for *S. aureus*; 4 to 8× the MIC of ampicillin, 1 to 2× the MIC of gentamicin, and 1 to 4× the MIC of ciprofloxacin for *E. coli*; and 4 to 8× the MIC of imipenem, 2 to 4× the MIC of tobramycin, and 1 to 4× the MIC of ciprofloxacin for *P. aeruginosa*.

Additional experiments exposing *E. coli* ATCC 25922 to gentamicin (1× the MIC) and ciprofloxacin (1 to 2× the MIC) and *P. aeruginosa* B 408 to imipenem (4 to 8× the MIC), tobramycin (1 to 2× the MIC), and ciprofloxacin (1 to 2× the MIC) for 2 h were performed at pHs 5 and 6.

The multiples of the MICs were based on the MICs at each individual pH. Unexposed control culture was employed at each pH level.

Determination of PAE. The PAEs were determined by standard methods (6). Drug removal after exposure was accomplished by a 10^{-2} or 10^{-3} dilution of exposed experiment or unexposed control culture into fresh warm MHB at each individual pH. In each experiment, an additional drug control was employed by inoculating similarly diluted unexposed organisms into fresh MHB, into which a 10^{-2} or 10^{-3} dilution of the highest drug concentration had been made. A drug control growing at the same rate as the unexposed control assured that the PAEs observed were not due to residual subinhibitory levels of drug. The initial experiments were conducted with a drug control at each pH level, but since no residual drug effects were seen in initial experiments, drug controls in later confirmatory experiments were only done at pH 7.4 (supplemented MHB).

Cultures were sampled prior to and immediately after drug exposure and removal and every 1.5 h thereafter until the culture tubes turned cloudy. Viability counts were determined by plating serial 10-fold dilutions of the samples on Mueller-Hinton agar and incubating for 18 to 24 h at 35.5°C. The PAE was calculated according to the equation $PAE = T - C$, where T is the time (in hours) required for the viability count (CFU per milliliter) in the test culture to increase 1 $\log_{10}$ above the count observed immediately after drug removal and where C is the time required for the count in an untreated control culture to increase by 1 $\log_{10}$ above the count observed immediately after the identical removal procedure.

Most experiments were repeated two to four times on different days.

Growth rate. The growth rates of unexposed organisms were determined in every PAE experiment at each pH level. It was calculated from 3 h of growth and expressed as $\Delta\log_{10}$ CFU per milliliter per hour.

Bactericidal rate. The bactericidal rate was determined after 1 h of drug exposure at all pHs as part of each PAE experiment. It was defined as the difference between the inoculum and the viable count immediately before drug removal and expressed as $\Delta\log_{10}$ CFU per milliliter per hour. Furthermore, the bactericidal activity (kill curves) was determined separately during continuous exposure of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* B 408 to the study drugs at the concentrations described above at each pH.

Statistics. The dependency of the PAE, growth, and bactericidal rate on pH were tested with a one-factor analysis of variance; the change in pH during 24 h of growth and the impact of Ca^{2+}-Mg^{2+} supplementation on the duration of the PAE and bactericidal rate were tested with Student's t test; and the relationship between the duration of the PAE and bactericidal rate at each individual pH level were tested by linear regression (StatView 512+; BrainPower Inc., Calabasas, Calif.). The level of significance was chosen at $\alpha = 0.05$.

RESULTS

Effect of pH. (i) MICs. The MICs at each pH for the test organisms are shown in Table 1. The antimicrobial agents were in general more active at alkaline than at acid pHs. The difference in MICs between pH 5.0 and 8.0 was most prominent for gentamicin and ciprofloxacin against *S. aureus* and *E. coli*, ranging from 32- to 267-fold (five to eight dilutions). This difference was four- to eightfold for tobramycin and ciprofloxacin against *P. aeruginosa* but was fourfold or less for the β-lactams.

(ii) Growth rates. The growth rates of the organisms were only minimally affected by pH (Fig. 1). However, a trend towards lower growth rate (<0.25 $\log_{10}$ CFU/ml per h) was observed at pHs 5 and 6 (P, not significant [NS]).

(iii) Bactericidal rates. The bactericidal rates after 1 h of exposure (Table 2) of the aminoglycosides and ciprofloxacin against *E. coli* and *P. aeruginosa* were, on the other hand, dependent on the pH. The initial bactericidal rate of imipenem for *P. aeruginosa* was likewise impaired at low pHs,

TABLE 1. MICs of drugs for test organisms at different pHs

Species and strain	Drug	MIC at pH:					
		5	6	7	7.4	8	7.4[a]
S. aureus ATCC 25923	Dicloxacillin	0.25	0.5	0.5	0.5	1	0.5
	Gentamicin	16	1	0.125	0.125	0.06	0.125
	Ciprofloxacin	4	0.5	0.25	0.5	0.5	0.25
E. coli							
ATCC 25922	Ampicillin	16	4	4	4	4	8
	Gentamicin	64	8	4	2	2	2
	Ciprofloxacin	1	0.125	0.03	0.03	0.03	0.015
B 5756	Gentamicin	64	8	4	2	2	1
	Ciprofloxacin	1	0.25	0.06	0.015	0.015	0.015
P. aeruginosa							
B 408	Imipenem	32	4	8	4	8	4
	Tobramycin	16	4	2	2	2	0.25
	Ciprofloxacin	1	1	0.5	0.5	0.25	0.25
ATCC 27853	Imipenem	16	4	4	4	4	4
	Ciprofloxacin	1	0.5	0.5	0.5	0.25	0.25

[a] MIC in nonsupplemented MHB at pH 7.4.

but to a relatively lesser degree. In contrast, neither the initial activity of ampicillin against *E. coli* nor the initial activity of any of the agents tested against *S. aureus* was dependent on pH. The subsequent bactericidal activity of all the antimicrobial agents studied was, however, significantly less at acid than at alkaline pHs as demonstrated by the $\Delta\log_{10}$ CFU per milliliter after 4 h of exposure in Table 2.

(iv) PAE. The duration of the PAE for the test organisms at different pHs is shown collectively in Table 3, and graphic examples are demonstrated in Fig. 2 through 4.

The PAE of *S. aureus* induced by dicloxacillin ranged from 1.9 to 2.9 h but was neither significantly nor predictably dependent on the pH. The duration of the PAEs induced by gentamicin and ciprofloxacin against *S. aureus* was, on the other hand, significantly pH dependent. The PAE of gentamicin was ~0.5 to 1.0 h longer at pH 5 than at more alkaline pHs. The PAEs produced by ciprofloxacin were, in some contrast, up to threefold longer at either end of the pH spectrum, pH 5 and pH 8, than at more intermediate pHs (Fig. 2).

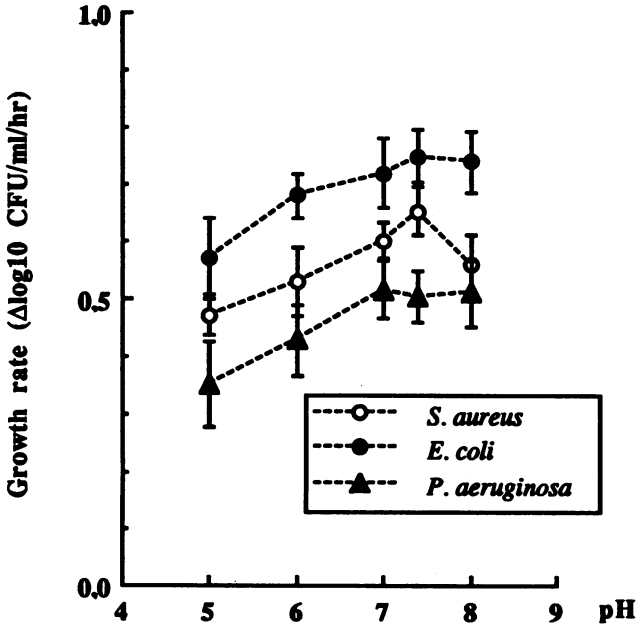

FIG. 1. Growth rates (untreated controls) at different pHs of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* B 408. Mean ± SD values from multiple experiments are represented by points with bars.

A stepwise increase in the duration of the PAE for *E. coli* after exposure to gentamicin and ciprofloxacin from pH 5 to 8 (Fig. 3; Table 3) was observed. For both agents, either an insignificant (<0.5 h) or no PAE was induced at pH 5. A PAE of ~1.0 h duration was reached at pH 7 with gentamicin and at pH 7.4 with ciprofloxacin. Determinations of PAEs induced by concentrations of these agents exceeding 2 to 4× the MIC were difficult or impossible because of rapid and complete microbial kills at higher concentrations. For the same reason, PAE determinations were difficult after longer than 1 h of exposure to the drug and were only successful after 2 h of exposure at acid pHs (pH 5 and 6). A prolongation of a mean ± the standard deviation (SD) of 0.3 ± 0.3 h at pH 5 and 0.1 ± 0.2 at pH 6 in the duration of the PAE was obtained after 2 h of exposure to gentamicin (1× the MIC) compared with the PAE after 1 h of exposure. Similarly, a prolongation of 0.6 ± 0.3 h and 0.7 ± 0.2 h was demonstrated after 2 h of exposure to ciprofloxacin (2× the MIC) at pHs 5 and 6, respectively. No PAE was induced by ampicillin against *E. coli* at any pH.

The PAEs for *P. aeruginosa* were markedly dependent on the pH (Fig. 4; Table 3). The PAE of imipenem against *P. aeruginosa* was 0.8 to 1.0 h shorter at pH 5 than at higher pHs. Similar to the observations with *E. coli*, tobramycin and ciprofloxacin induced only an insignificant or no PAE against *P. aeruginosa* at pH 5. A PAE of ~1.0 h was obtained at pH 6 with tobramycin, but only at pH 7 to 7.4 with ciprofloxacin. Again, exposure times exceeding 1 h proved difficult and indeed impossible at pH 7 or higher. There was prolongation of the PAE duration (mean ± SD) of 0.8 ± 0.2 h and 0.4 ± 0.3 h after 2 h of exposure to imipenem (4× the MIC), of 0.7 ± 0.3 h and 0.2 ± 0.2 h after exposure to tobramycin (2× the MIC), and 0.0 ± 0.3 h and 0.4 ± 0.3 h after exposure to ciprofloxacin (2× the MIC) at pHs 5 and

TABLE 2. Bactericidal activity after 1 and 4 h of exposure of the study drugs at different pHs against *S. aureus*, *E. coli*, and *P. aeruginosa*

Organism, drug, and time (h)	MIC (fold)	Supplemented MHB $\Delta\log_{10}$ CFU/ml (mean ± SD) at pH[a]: 5	6	7	7.4	8	*P*	Nonsupplemented MHB ($\Delta\log_{10}$ CFU/ml [mean ± SD] at 7.4)[b]
S. aureus ATCC 25923								
Dicloxacillin	4							
1		−0.4 ± 0.2	−0.2 ± 0.1	−0.2 ± 0.2	−0.3 ± 0.1	−0.3 ± 0.2	NS	−0.4 ± 0.3
4		0.0 ± 0.2	−0.1 ± 0.2	−0.8 ± 0.2	−1.1 ± 0.3	−0.9 ± 0.3	0.001	−0.8 ± 0.2
Gentamicin	2							
1		0.1 ± 0.2	−0.2 ± 0.2	0.0 ± 0.2	−0.2 ± 0.2	−0.4 ± 0.3	NS	−0.6 ± 0.3
4		−0.4 ± 0.3	−1.1 ± 0.4	−1.4 ± 0.3	−1.9 ± 0.2	−1.7 ± 0.3	0.001	−2.2 ± 0.2
Ciprofloxacin	2							
1		−0.5 ± 0.3	−0.3 ± 0.2	−0.6 ± 0.4	−0.4 ± 0.1	−0.4 ± 0.2	NS	−0.8 ± 0.4
4		−0.2 ± 0.4	−0.4 ± 0.3	−0.9 ± 0.5	−1.5 ± 0.3	−2.6 ± 0.4	0.001	−1.6 ± 0.2
E. coli ATCC 25922								
Ampicillin	4							
1		−0.4 ± 0.2	−0.4 ± 0.3	−0.2 ± 0.1	−0.3 ± 0.3	−0.3 ± 0.1	NS	−0.2 ± 0.3
4		−2.1 ± 0.3	−2.6 ± 0.2	−3.5 ± 0.4	−4.2 ± 0.3	−4.1 ± 0.3	0.001	−4.6 ± 0.4
Gentamicin	2							
1		−0.7 ± 0.2	−1.2 ± 0.3	−3.0 ± 0.4	−2.9 ± 0.4	−2.9 ± 0.3	0.001	−2.3 ± 0.2
4		−1.8 ± 0.3	−2.5 ± 0.3	>−5.0	>−5.0	>−5.0	0.001	−4.4 ± 0.4
Ciprofloxacin	2							
1		−0.4 ± 0.2	−1.0 ± 0.3	−0.9 ± 0.1	−2.7 ± 0.2	−2.8 ± 0.3	0.001	−2.4 ± 0.3
4		−2.6 ± 0.3	−3.6 ± 0.3	−4.9 ± 0.2	−4.8 ± 0.4	−4.7 ± 0.4	0.001	−4.2 ± 0.4
P. aeruginosa B 408								
Imipenem	4							
1		−0.4 ± 0.2	−0.9 ± 0.3	−0.8 ± 0.3	−0.9 ± 0.2	−1.3 ± 0.4	0.02	−1.0 ± 0.4
4		−0.8 ± 0.2	−1.4 ± 0.2	−2.0 ± 0.2	−1.8 ± 0.1	−2.1 ± 0.3	0.001	−1.7 ± 0.2
Tobramycin	4							
1		−0.3 ± 0.5	−1.8 ± 0.6	−3.1 ± 0.3	−3.4 ± 0.1	−3.4 ± 0.4	0.001	−3.1 ± 0.4
4		−2.4 ± 0.4	−2.9 ± 0.6	−4.3 ± 0.3	−4.1 ± 0.3	−3.8 ± 0.3	0.001	−3.7 ± 0.4
Ciprofloxacin	2							
1		0.1 ± 0.2	−2.4 ± 0.1	−3.2 ± 0.2	−3.1 ± 0.2	−3.0 ± 0.5	0.001	−2.8 ± 0.3
4		−0.4 ± 0.3	−2.8 ± 0.2	−3.8 ± 0.2	−4.7 ± 0.2	−4.3 ± 0.5	0.001	−4.3 ± 0.4

[a] Bactericidal rates of other drug concentrations paralleled those demonstrated here and are not shown.
[b] Bactericidal activity in nonsupplemented MHB at pH 7.4.

6, respectively. The differences at lower drug concentrations were even less.

No correlation was demonstrated between the duration of the PAE and the initial bactericidal rate after 1 h of exposure at each individual pH level, whether examined for the entire data base or each organism and individual antimicrobial group separately (the Pearson's correlation coefficients [*r*] for the three bacterial species and all antimicrobial agents at pHs 5, 6, 7, 7.4, and 8 being, for example, 0.007, 0.096, 0.072, 0.040, and 0.068, respectively [*P*, NS]).

(v) Change in pH. The pH at each level did not change perceptively during each experiment, as measured after 3 to 4.5 h for controls and 6 to 8 h for exposed organisms. The mean ± SD change in the pH of the *P. aeruginosa* cultures was 0.05 ± 0.14, that of *S. aureus* cultures was 0.09 ± 0.03, and that of *E. coli* cultures was 0.23 ± 0.14. However, after 24 h of growth, significant changes in the pH had occurred. The pHs of the *S. aureus*, the *E. coli*, and the *P. aeruginosa* cultures had converged towards 6, ~6.5, and 7, respectively.

Effect of Ca^{2+} and Mg^{2+} supplementation. (i) Concentrations. The concentration of Ca^{2+} in equally supplemented broth at each pH ranged from 55.6 to 57.2 mg/liter (1.39 to 1.43 mmol/liter), and the concentration of Mg^{2+} ranged from 23.8 to 24.5 mg/liter (0.99 to 1.02 mmol/liter). The free Ca^{2+} concentration ranged from 50 mg/liter (1.25 mmol/liter) at pH 5 to 37.6 mg/liter (0.94 mmol/liter) at pH 8. In nonsupplemented broth at pH 7.4, the total Ca^{2+} concentration measured 7.2 mg/liter (0.18 mmol/liter) and total Mg^{2+} concentration measured 4.1 mg/liter (0.17 mmol/liter).

(ii) MICs. The MICs at pH 7.4 determined in supplemented versus nonsupplemented MHB did not differ by more than one dilution, except for that of tobramycin for *P. aeruginosa*, in which case the drug was eightfold less active in supplemented MHB (Table 1).

(iii) Growth and bactericidal rates. Growth rates were not dependent on the cationic concentrations studied (data not shown). Similarly, the mean bactericidal activity at pH 7.4 (Table 2) differed by <0.1 $\log_{10}$ CFU/ml after 1 h of exposure and by ≤ 0.5 $\log_{10}$ CFU/ml after 4 h of exposure between the two cationic concentrations studied (*P*, NS).

(iv) PAEs. The difference between the PAEs in Ca^{2+}-Mg^{2+}-supplemented and nonsupplemented MHB at pH 7.4 never exceeded 0.4 h (Table 3). The mean difference was <0.1 h (*P*, NS).

DISCUSSION

Several factors that have been shown to influence the presence and duration of the PAE include the type of organism, the class and concentration of antibiotic, the

TABLE 3. Duration of the PAE at different pHs for *S. aureus*, *E. coli*, and *P. aeruginosa*

Organism and drug (MIC, fold)	Supplemented MHB						Nonsupplemented MHB (PAE duration [h] at pH 7.4 [mean ± SD])[a]
	PAE duration (h) (mean ± SD) at pH:					P	
	5	6	7	7.4	8		
S. aureus ATCC 25923							
Dicloxacillin							
4	1.9 ± 0.1	2.5 ± 0.1	1.9 ± 0.3	1.7 ± 0.1	2.6 ± 0.2	0.07	1.7 ± 0.3
8	2.6 ± 0.3	2.9 ± 0.2	2.7 ± 0.4	2.3 ± 0.3	2.9 ± 0.3	0.20	2.5 ± 0.2
Gentamicin							
1[b]	0.6	0.4	0.3	0.5	0.2		0.6
2	1.4 ± 0.1	0.7 ± 0.5	0.6 ± 0.2	1.0 ± 0.1	0.6 ± 0.1	0.01	1.2 ± 0.2
Ciprofloxacin							
1[b]	1.1	0.3	0.5	1.0	2.2		0.8
2	2.1 ± 0.1	0.9 ± 0.3	0.9 ± 0.2	1.7 ± 0.1	3.1 ± 0.2	0.003	1.4 ± 0.2
4	2.7 ± 0.4	1.6 ± 0.5	1.0 ± 0.4	2.4 ± 0.5	3.2 ± 0.3	0.01	2.1 ± 0.3
E. coli ATCC 25922							
Ampicillin							
4	−0.5 ± 0.2	−0.2 ± 0.3	−0.2 ± 0.4	−0.1 ± 0.3	−0.2 ± 0.3	0.2	−0.2 ± 0.3
8[b]	−0.4	−0.2	−0.3	−0.5	−0.3		−0.2
Gentamicin							
1	0.2 ± 0.2	0.5 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	1.0 ± 0.3	0.0004	0.9 ± 0.2
2	0.5 ± 0.1	0.8 ± 0.3	1.0 ± 0.2	0.9 ± 0.2	1.3 ± 0.2	0.0002	1.1 ± 0.4
Ciprofloxacin							
1	0.1 ± 0.2	0.3 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.009	0.9 ± 0.4
2	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	1.1 ± 0.1	0.02	1.1 ± 0.3
4[b]	0.5	0.4	0.8	1.4	1.3		1.2
B 5756							
Gentamicin							
1[b]	−0.1	0.4	0.5	0.8	0.8		0.9
2[b]	0.6	1.0	1.1	1.0	1.3		1.3
Ciprofloxacin							
2[b]	0.6	0.9	1.2	1.4	1.2		1.0
P. aeruginosa							
B 408							
Imipenem							
4	1.0 ± 0.2	1.8 ± 0.3	2.3 ± 0.3	2.1 ± 0.4	2.2 ± 0.2	0.007	2.4 ± 0.3
Tobramycin							
2	0.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.5	1.9 ± 0.1	0.01	1.5 ± 0.5
4	0.5 ± 0.2	1.4 ± 0.1	1.6 ± 0.1	2.0 ± 0.3	2.1 ± 0.4	0.007	2.1 ± 0.6
Ciprofloxacin							
1	0.1 ± 0.2	0.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.2	0.9 ± 0.1	0.0001	0.9 ± 0.3
2	0.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	1.0 ± 0.3	0.9 ± 0.2	0.01	1.1 ± 0.1
4[b]	0.5	0.5	1.1	1.4	0.9		1.6
ATCC 27853							
Imipenem							
4	1.1 ± 0.1	2.1 ± 0.1	2.5 ± 0.3	2.3 ± 0.2	2.3 ± 0.1	0.0005	2.1 ± 0.4
8	1.4 ± 0.1	2.2 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	2.6 ± 0.1	0.02	2.5 ± 0.2
Ciprofloxacin							
1	−0.3 ± 0.2	0.1 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.0001	1.2 ± 0.2
2[b]	−0.1	0.1	1.0	1.1	1.4		1.1

[a] PAEs in nonsupplemented MHB at pH 7.4.
[b] Data based on 1 or 2 experiments only.

duration of exposure to the antibiotic, combinations of antibiotics, the size of inoculum, and the type of medium (6).

The results of this study indicate that the pH of the medium may significantly influence the duration of the PAE as well.

The PAEs of tobramycin, ciprofloxacin, and imipenem for *P. aeruginosa* and of gentamicin and ciprofloxacin for *E. coli* were significantly shorter at acid pH than at alkaline pH, even when corrected for differences in the MIC. PAEs of potential significance (i.e., ≥1 h) were generally not obtained until the pH reached 7 to 7.4. PAEs of ampicillin for *E. coli* were not induced at any pH. The PAEs induced by gentamicin and ciprofloxacin against *S. aureus* were also dependent on the pH, but in a different fashion. The PAE produced by gentamicin was longer at pH 5 than at other pHs, whereas the PAEs exhibited after ciprofloxacin were two- to threefold longer at pHs 5 and 8 than at pH 7. In contrast, the PAE exhibited by dicloxacillin against *S. aureus* was not predictably dependent on the pH.

The short duration of the PAEs for *E. coli* and *P. aeru-*

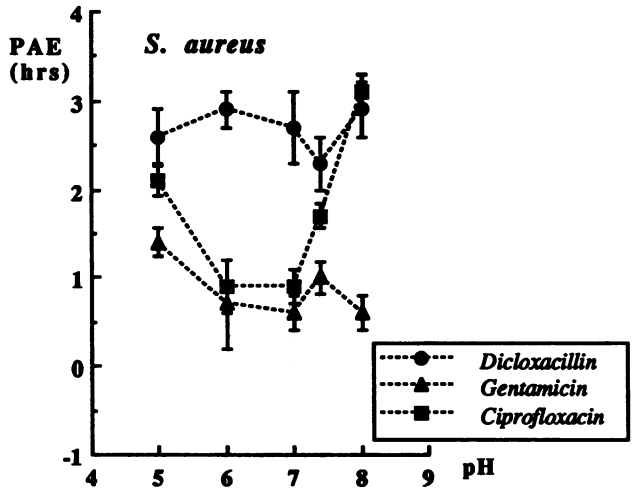

FIG. 2. PAEs at different pHs for *S. aureus* ATCC 25923 after 1 h of exposure to dicloxacillin (8× the MIC), gentamicin (2× the MIC), and ciprofloxacin (2× the MIC). Mean ± SD values from multiple experiments are represented by points with bars.

ginosa at low pHs could only be partially overcome by a longer exposure time of 2 h. However, the information on this issue is incomplete, since the duration of PAEs with exposure times longer than 1 h at higher pHs (7, 7.4, and 8) was impossible to determine because of more rapid and often nearly complete bactericidal activity at those levels.

The PAEs in these studies were all induced at equipotent drug concentrations at each individual pH, i.e., the concentrations (in multiples of MICs) were corrected for differences in the MIC. Furthermore, the pHs remained stable throughout each experiment.

Other investigators (4, 5, 9) have shown variable effects of urine at pH 5.5 on the duration of the PAE induced by

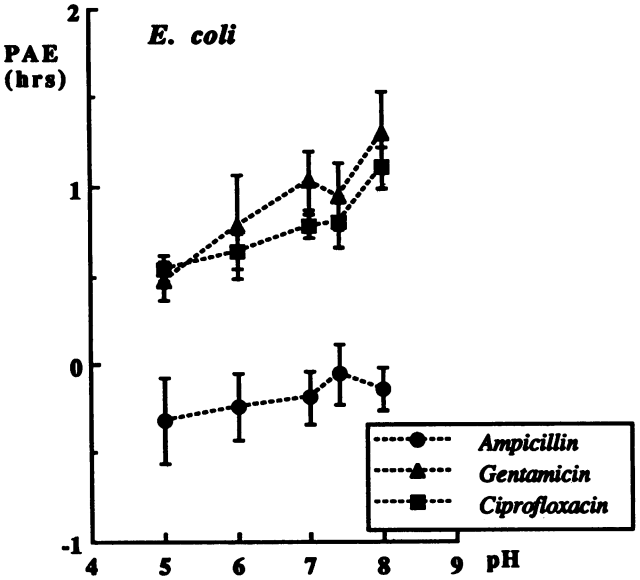

FIG. 3. PAEs at different pHs for *E. coli* ATCC 25922 after 1 h of exposure to ampicillin (4× the MIC), gentamicin (2× the MIC), and ciprofloxacin (2× the MIC). Mean ± SD values from multiple experiments are represented by points with bars.

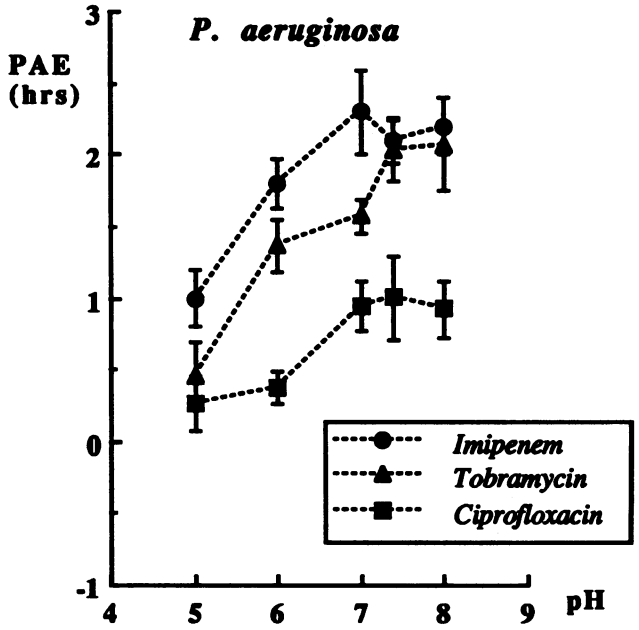

FIG. 4. PAEs at different pHs for *P. aeruginosa* B 408 after 1 h of exposure to imipenem (4× the MIC), tobramycin (4× the MIC) and ciprofloxacin (2× the MIC). Mean ± SD values from multiple experiments are represented by points with bars.

various quinolones for several gram-negative bacilli compared with the PAE in broth at pH 7.4. However, in those studies, the authors used in vitro concentrations in urine that were 80- to 100-fold higher than those in broth. In studies with antifungal agents and yeasts (24), a longer duration of PAE was observed with 5-fluorocytosine in yeast nitrogen base (pH 5.5) than in buffered yeast nitrogen base (pH 7.0) against *Candida albicans*.

As a prerequisite for and part of the PAE experiments, the impact of the pH on other parameters, i.e., growth rate, MICs, and bactericidal rate, was examined as well. Bactericidal activity was further studied separately by kill curves. Growth rates of the organisms studied were not significantly affected by the pH, although a trend towards a lower rate was observed at acid pHs. The effect on the PAE by different pHs was thus primarily through influence on the duration of regrowth (T in the above equation), rather than on growth rates of unexposed controls (C). In contrast, the MICs and bactericidal activity (shown after 1 and 4 h of drug exposure in Table 2) were significantly dependent on the pH. An acid pH had in general a deleterious effect on the MICs of the agents used in the study. The initial bactericidal activity (at the time of drug removal in the PAE experiments) of the aminoglycosides and ciprofloxacin against *E. coli* and *P. aeruginosa* was similarly 1 to 3 $\log_{10}$ CFU/ml per h slower at acid pHs than at more alkaline pHs. On the other hand, the initial bactericidal activity of imipenem against *P. aeruginosa* was much less affected by the pH and that of ampicillin against *E. coli* was not affected at all. The initial bactericidal activity of the agents tested against *S. aureus* did not depend on the pH. However, and more importantly, the subsequent bactericidal activity of all the antimicrobial agents tested, as determined by continuous kill curves, was significantly pH dependent, being 1 to 4 $\log_{10}$ CFU/ml less at acid pHs than at more alkaline pHs.

Although previous information on the impact of the pH on

the PAE is limited, ample data in the literature on the influence of pH on antibacterial inhibitory and bactericidal activity are available. As an example, an acid pH in general adversely affects the MIC and MBC of aminoglycosides (1, 18), quinolones containing a piperazinyl side chain (9, 20, 27, 28), imipenem (16), and others (18).

The explanation for these differences is not clear but is probably related to changes in permeability or binding of antimicrobial agents to surface or intracellular receptors as a result of varying degrees of ionization of drugs and cellular structures with different pK_as (17, 18, 23), resulting, perhaps, in less structural damage at low pHs after exposure to some drugs, thus allowing more rapid regrowth. For example, impaired energy-dependent aminoglycoside transport across bacterial cell walls has been demonstrated at low pHs because of the diminution of electrical potential [Δ(pounds per square inch)] in an acid external environment (7, 8). Similar processes are probably responsible for the impact of the pH on the PAE and may actually be variable according to the bacterial species and the antimicrobial agent. These processes may, however, be difficult to elucidate, since the mechanism(s) of the PAE remains unknown, but studies on bacterial metabolism and ultrastructural changes at different pHs might provide further insight into this matter.

Calcium and magnesium were added to the culture medium according to standard recommendations (22), and the resulting measured concentrations of Ca^{2+} and Mg^{2+} were within reasonable proximity of the cation concentrations reported in abscess fluid (2, 3). The cation supplementation of the medium at pH 7.4 influenced neither the duration of the PAE nor the bactericidal rate. However, in concordance with previous studies (12–14, 25), the cations interfered with the action of tobramycin against *P. aeruginosa* and increased the MIC by eightfold.

As previously mentioned, the pH of the culture medium did not change perceptively during the course of each experiment. However, when the culture tubes were incubated for 24 h, the pH of the medium converged towards pH 6, 6.5, or 7 for *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. This perhaps illustrates the impact of the fermentative capacity of the microorganisms and the buffering action of their metabolites in the generation of the specific acid environment of infected foci.

In conclusion, an acid pH has in general a deleterious effect on the activity of aminoglycosides and ciprofloxacin against *E. coli* and *P. aeruginosa*, with the MIC being higher, the bactericidal rate being lower, and the PAE being shorter at pH 5 (and to a lesser extent at pH 6) than at more alkaline pHs. Similar results for imipenem against *P. aeruginosa* were observed. The bactericidal activities of ampicillin against *E. coli* and all three agents tested against *S. aureus* were likewise pH dependent. On the other hand, the PAEs induced by ampicillin against *E. coli* and dicloxacillin against *S. aureus* were not predictably dependent on the pH, whereas the PAEs induced by ciprofloxacin against *S. aureus* were longest at either end of the pH spectrum. Since the pH in abscess cavities may be close to 5, these observations may be of importance for employment of the agents studied in closed or poorly drained infections. Clearly, further experiments in vivo are needed to confirm these results.

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