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Post-antibiotic effect of three quinolones against Gram negative isolates from urine

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The post-antibiotic effect (PAE) is defined as the bacterial growth suppression which persists after a limited exposure to an antimicrobial agent. The PAE and the bactericidal effect of the quinolones ciprofloxacin, norfloxacin and nalidixic acid have been studied against several urinary isolates of Gram-negative bacteria. The PAE was determined after one hour's exposure to the antimicrobial agent using an initial inoculum of 10^5 to 10^6 cfu/ml; the drug was rapidly removed by a 10^{-2} dilution technique in antibiotic-free medium. When ciprofloxacin was used at four times its MIC the PAEs were 1.37 ± 0.09; 2.45 ± 0.63 and 2.86 ± 0.15 h against *Esch. coli*, *Klebs. pneumoniae* and *Pseudomonas aeruginosa*, respectively. We found lower values for norfloxacin under the same conditions, and nalidixic acid did not induce a significative PAE. These results could support changes in dosing intervals of norfloxacin and ciprofloxacin, with possibly greater intervals between doses.

Keywords: Postantibiotic effect. Quinolones. Gram-negative bacteria.

Introduction

Infectious disease treatment protocols are currently stabilised to assure greater antimicrobial levels (blood or action site levels) than the drug's MIC. Consequently, dosing intervals should not exceed the time that anti-microbial levels are maintained in order to inhibit bacterial growth. This means that the dosing interval of the anti-microbial agent is based on its rate of elimination; other pharmacokinetic parameters such as absorption, protein binding, metabolism and tissue perfusion, should also be considered.[1]

Although post-antibiotic effect (PAE) is not a new parameter, it is not usually considered when establishing antibiotic therapy. PAE describes the persistent suppression of bacterial growth after a brief exposure to supra-inhibitory concentrations of anti-microbial agents.[2]

PAE was first observed by Bigger in 1944, for penicillin,[3] but its study was not resumed until during the last 10 years, when Bigger's initial studies were continued by other authors.[4,5] *In vitro* and *in vivo* demonstrations of PAE[6] would mean that even when anti-microbial levels had fallen below the MIC it would still suppress bacterial growth, thus extending its activity.

The exact mechanism underlying PAE is unknown. Other authors[2] have proposed that PAE would be due to site-action anti-microbial persistence and/or to a non-lethal bacterial damage. The biological significance of PAE is not known. McDonald *et al.*[4] and Pruul *et al.*[7] have observed this effect in animal model infections, and have been able to demonstrate that PA-phase bacteria are more susceptible to phagocytosis.

It has been demonstrated recently that some quinolones exert PAE against certain Gram-positive and Gram-negative bacteria.[8–10] In this paper we present an evaluation of the killing curve and the PAE of three quinolones—nalidixic acid, norfloxacin, and ciprofloxacin—against *Esch. coli*, *Klebs. pneumoniae* and *Ps. aeruginosa* isolated from urine. We have also studied the influence of anti-microbial concentration and exposure time on these parameters.

Materials and methods

Micro-organisms. Four clinical isolates of *Esch. coli*, four of *Klebs. pneumoniae* and four of *Ps. aeruginosa* were used. The bacteria were isolated

from patients with symptomatic urinary infections at the Microbiology Department of the University Hospital of Granada.

Anti-microbials. Three quinolones were studied: nalidixic acid (Sigma), a classic quinolone; norfloxacin (Merck) and ciprofloxacin (Bayer), two fluorated quinolones. Several concentrations (MIC, 2, 4 and 8 times the MIC) were tested.

Determination of MIC. The Mueller-Hinton agar double dilution method was used.[11]

Determination of the bactericidal effect: killing curve. The initial inoculum, a 10^6 to 10^7 cfu/ml logarithmic phase culture, was exposed to the anti-microbial and incubated at 37°C with agitation for 8 h in Schaedler broth (Oxoid). Bacterial viability was determined 30 min after exposure, and after each hour up to 8 h, by growth in Mueller-Hinton agar (Oxoid). The killing curve was derived by relating viable bacteria numbers *per* ml to exposure times. The bactericidal effect was calculated as the difference in the number of viable bacteria between a control culture (without added anti-microbial) and a treated culture related to the exposure time and expressed as cfu/ml.

Determination of the post-antibiotic effect. Initial conditions were the same as for determination of the bactericidal effect. The anti-microbials were removed after 30, 60 and 120 min exposure to *Esch. coli* HC-5, *Klebs. pneumoniae* HC-776 and *Ps. aeruginosa* HC-46 strains, and after 60 min for the other strains tested. Removal was performed by a 10^{-2} dilution in antibiotic-free medium. (Our experience shows that this dilution is sufficient to remove moderate concentrations of the anti-microbials.) Viable bacterial counts were made every hour, up to 8 h, after removal. Quantification of the PAE was performed as described by Craig and Gudmunson[2] as the difference in time required by test and control cultures to increase one $\log_{10}$ cfu/ml after the antibiotic was removed.

Results

The *in vitro* activity of nalidixic acid, norfloxacin and ciprofloxacin against the various bacteria is shown in Table 1. Two strains of *Ps. aeruginosa* showed high resistance against nalidixic acid, and were not studied further.

Results obtained from killing curves showed a high and intense bactericidal effect for ciprofloxacin. After one hour's exposure, bactericidal effects for *Esch. coli*, *Klebs. pneumoniae* and *Ps. aeruginosa* were 5.37, 5.20 and 4.07 $\log_{10}$ cfu/ml respectively. The bactericidal effect was slightly lower for norfloxacin, and clearly lower for nalidixic acid (less than 2 $\log_{10}$ cfu/ml—Table 2).

Table 1. MICs of nalidixic acid, norfloxacin and ciprofloxacin against Gram negative isolates

Microorganism	MIC (mcg/ml)		
	Nalidixic acid	Norfloxacin	Ciprofloxacin
Esch. coli			
HC-5	2	0.125	0.03
HC-9	2	0.015	0.007
HC-11	2	0.03	0.007
HC-14	2	0.03	0.007
Klebs. pneumoniae			
HC-791	2	0.03	0.03
HC-776	2	0.125	0.03
HC-712	2	0.5	0.06
HC-257	2	0.125	0.25
Ps. aeruginosa			
HC-40	128	1.0	0.25
HC-46	128	0.5	0.25
HC-58	128	0.5	0.125
HC-60	128	1.0	0.25

Table 2. Bactericidal effect of three quinolones against *Esch. coli*, *Klebs. pneumoniae* and *Ps. aeruginosa*

Anti-microbial	Microorganism	Exposure time 0.5 h	1 h	2 h	3 h	4 h	5 h
Nalidixic acid	*Esch. coli*	0.57 ± 0.08	1.32 ± 0.40	4.72 ± 0.59	5.92 ± 0.15	6.45 ± 0.27	6.92 ± 0.43
	Klebs. pneumoniae	0.77 ± 0.11	1.55 ± 0.16	4.12 ± 0.75	5.30 ± 0.81	5.9 ± 0.72	6.3 ± 0.83
Norfloxacin	*Esch. coli*	1.25 ± 0.38	2.37 ± 0.76	4.0 ± 0.57	5.15 ± 0.47	5.52 ± 0.43	5.85 ± 0.48
	Klebs. pneumoniae	2.42 ± 0.22	4.3 ± 0.31	5.95 ± 0.11	6.9 ± 0.27	7.77 ± 0.50	8.55 ± 0.75
	Ps. aeruginosa	1.62 ± 0·11	3.67 ± 0.33	5.50 ± 0.90	6.65 ± 0.86	7.55 ± 0.61	8.50 ± 0.66
Ciprofloxacin	*Esch. coli*	2.65 ± 0.11	5.37 ± 0.33	6.62 ± 0.41	7.72 ± 0.64	n.d.	n.d.
	Klebs. pneumoniae	2.37 ± 0.40	5.20 ± 0.22	6.6 ± 0.57	7.37 ± 0.97	n.d.	n.d.
	Ps. aeruginosa	1.95 ± 0.35	4.07 ± 0.91	6.32 ± 0.41	7.30 ± 0.43	8.35 ± 0.47	9.52 ± 0.66

Results are expressed as the mean + SD for the four strains exposed to four times the MIC.

The PAE induced by the three quinolones against *Esch. coli*, *Klebs. pneumoniae* and *Ps. aeruginosa* strains is shown in Table 3. There was no significant difference in the PAE value for different strains of the same species. We found good activity for ciprofloxacin, but only obtained statistical differences for *Klebs. pneumoniae* ($P < 0.05$).

The influence of exposure time and antimicrobial concentration upon the PAE was studied for the following strains: *Esch. coli* HC-5, *Klebs. pneumoniae* HC-774 and *Ps. aeruginosa* HC-46. MIC, and MIC × 2, × 4 tested after 30, 60 and 120 min exp 3). There was a high concentratio for norfloxacin and ciprofloxacin

Table 3. Post-antibiotic effect of nalidixic acid, norfloxacin and ciprofloxacin against Gram negative isolates

Micro-organism	PAE (h) Nalidixic acid	Norfloxacin	Ciprofloxacin
Esch. coli			
HC-5	0.5	2.2	1.5
HC-9	0.7	1.0	1.25
HC-11	0.5	1.5	1.4
HC-14	0.7	1.25	1.33
M ± D.E.	0.60 ± 0.10	1.49 ± 0.45[a]	1.37 ± 0.09[a]
Klebs. pneumoniae			
HC-776	0.6	1.3	2.4
HC-712	0.3	1.6	2.0
HC-257	0.4	1.5	3.5
HC-791	0.4	1.7	1.9
M ± D.E.	0.42 ± 0.11	1.52 ± 0.15[a]	2.45 ± 0.63[a,b]
Ps. aeruginosa			
HC-40		1.5	2.1
HC-46		3.1	2.8
HC-58		2.8	3.66
HC-60		2.4	2.9
M ± D.E.		2.45 ± 0.60	2.86 ± 0.55

[a] Statistical significance from nalidixic acid ($P < 0.05$). [b] Statistical significance from norfloxacin ($P < 0.05$).

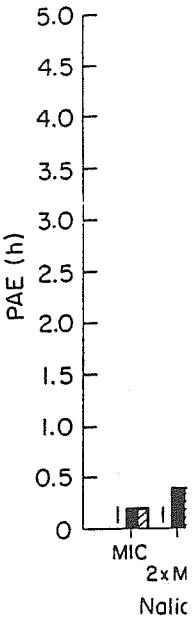

Fig. 1. Exposure time influence *coli* HC-5. (1) No PAE after 30

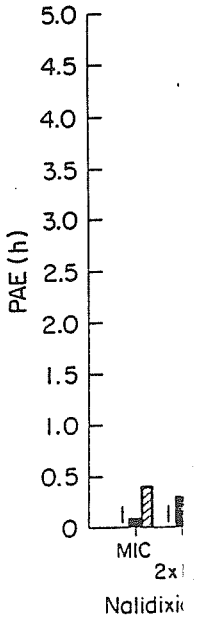

Fig. 2. Exposure time influen *Klebs. pneumoniae* HC-776. (1)

umoniae and *Ps. aeruginosa*

time

3 h	4 h	5 h
5.92 ± 0.15	6.45 ± 0.27	6.92 ± 0.43
5.30 ± 0.81	5.9 ± 0.72	6.3 ± 0.83
5.15 ± 0.47	5.52 ± 0.43	5.85 ± 0.48
6.9 ± 0.27	7.77 ± 0.50	8.55 ± 0.75
5.65 ± 0.86	7.55 ± 0.61	8.50 ± 0.66
7.72 ± 0.64	n.d.	n.d.
7.37 ± 0.97	n.d.	n.d.
7.30 ± 0.43	8.35 ± 0.47	9.52 ± 0.66

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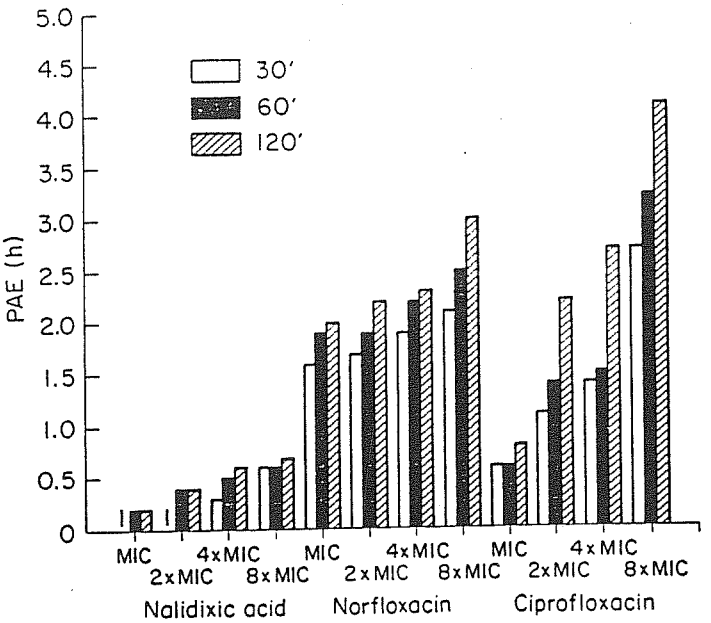

Fig. 1. Exposure time influence on PAE of nalidixic acid, norfloxacin and ciprofloxacin against *Esch. coli* HC-5. (1) No PAE after 30 min.

HC-46. MIC, and MIC × 2, × 4 and × 8 were tested after 30, 60 and 120 min exposure (Figs 1–3). There was a high concentration dependence for norfloxacin and ciprofloxacin, but not for nalidixic acid. Exposure time was found to prolong PAE for all the quinolones tested except for nalidixic acid, probably due to the insignificant PAE induced by this quinolone. We wish to

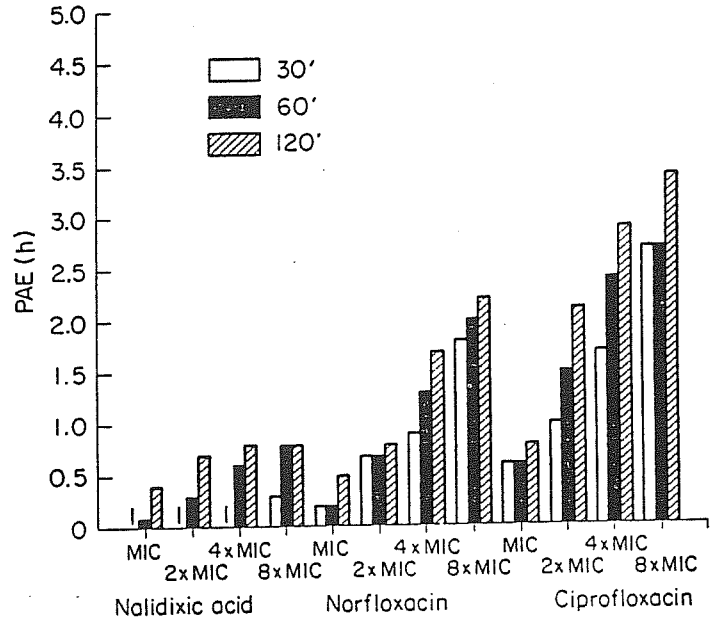

Fig. 2. Exposure time influence on PAE of nalidixic acid, norfloxacin and ciprofloxacin against *Klebs. pneumoniae* HC-776. (1) No PAE after 30 min.

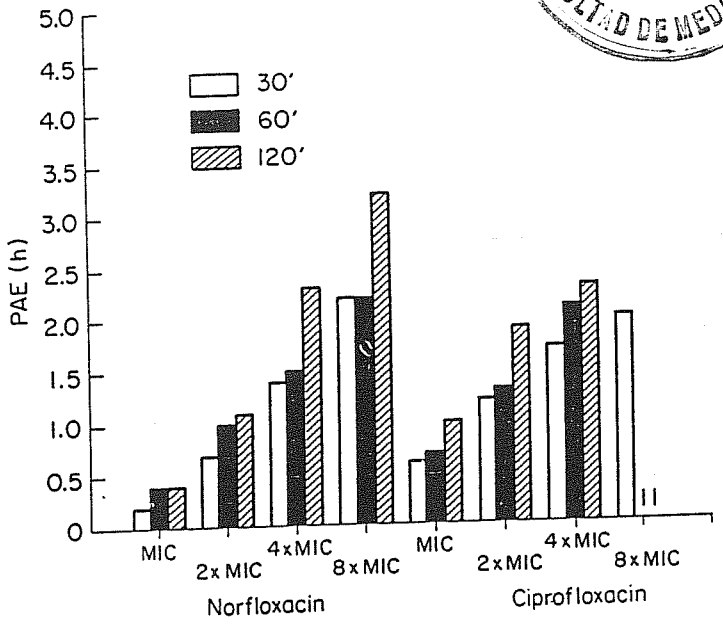

Fig. 3. Exposure time influence on PAE of nalidixic acid, norfloxacin and ciprofloxacin against *Ps. aeruginosa* HC-46. (1) PAE not determined.

emphasize the high PAE induced by norfloxacin against *Esch. coli* HC-5 at low concentrations (MIC and MIC × 2). In this case we noted less influence between PAE and exposure time. Due to the rapid bactericidal effect shown by ciprofloxacin against *Ps. aeruginosa* HC-46 when used at MIC × 8, the PAE could not be determined when exposure time exceeded 30 min.

Discussion

This study shows that ciprofloxacin and norfloxacin produce a significant PAE with *Esch. coli*, *Klebs. pneumoniae* and *Ps. aeruginosa* with a recuperation time between 1.37 and 2.86 h, depending on the microorganism-antimicrobial pair, when the drug was used at four times the MIC. This recuperation time was similar to results obtained by other authors for ciprofloxacin against *Staph. aureus*,[9] *Esch. coli* and *Ps. aeruginosa*.[2,8] PAE induced with these two fluorated quinolones was similar to that found by other authors for ofloxacin[12] and for anti-microbials that act on protein synthesis such as chloramphenicol and tetracicline.[4,13] It was also superior to that PAE induced by B-lactamics.[2,14] Nalidixic acid, however, induced a very little PAE (less than 0.5 h) under the same conditions.

Ciprofloxacin and norfloxacin produced PAE with MIC, unlike other anti-microbials that need high concentrations (MIC × 4, × 8, or higher). These effects have also been shown by Minguez[12] for ofloxacin.

PAE concentration dependence found for the three quinolones studied, without finding a maximum effect, leads us to think that PAE duration could increase even more when the concentration increases. The low MIC of quinolones against bacteria, and the higher concentrations found in patients (approx. 300 mcg/ml in the urine, and 3 mcg/ml in serum, in ciprofloxacin) would involve a greater PAE for quinolones—including nalidixic acid—that could considerably prolong their action *in vivo*.

The PAE would have an impact on anti-microbial dosing intervals, so that anti-microbials with a prolonged PAE (such as aminoglycosides and quinolones) would permit a greater interval between doses than other drugs with very little PAE, and without prejudice to treatment efficacy.

The understanding of anti-microbial PAE could end the controversy about whether it is better to administer the anti-microbial so that high concentrations are found in the organism for a shorter period, or moderate concentrations persisting throughout the whole treatment. High PAE anti-microbials could be given at high doses widely separated in on pharmacokinetics and PAI

Our conclusion is that a co PAE is becoming more nece antibiotic therapy. For norfl loxacin, the fluorated quinc longed PAE would justify between doses. Further studi to validate this effect.

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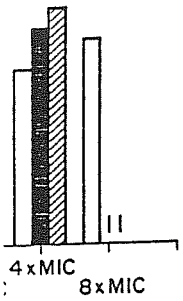

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high doses widely separated in time (depending on pharmacokinetics and PAE magnitude).

Our conclusion is that a consideration of the PAE is becoming more necessary for rational antibiotic therapy. For norfloxacin and ciprofloxacin, the fluorated quinolones, their prolonged PAE would justify a greater interval between doses. Further studies should be made to validate this effect.

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