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Bactericidal mechanism of gatifloxacin compared with other quinolones

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The quinolones differ in their mechanisms of bacterial killing. The rate of bacterial killing by quinolones can be influenced by the addition of bacterial protein or RNA synthesis inhibitors, and the growth phase of the bacterium. In this study, we compared the killing activities of gatifloxacin, trovafloxacin, ciprofloxacin and norfloxacin against staphylococci, pneumococci and *Escherichia coli.* Gatifloxacin killing of these organisms occurred regardless of the metabolic state of the microbes. Unlike the comparator quinolones, gatifloxacin killing was not influenced by the addition of bacterial protein or RNA synthesis inhibitors. Gatifloxacin was able to kill non-dividing staphylococcal and *E. coli* cells.

Introduction

Quinolones are bactericidal agents. They kill bacteria more rapidly than other antimicrobial bactericidal classes.[1] Quinolones exhibit concentration-dependent killing kinetics, with maximum killing rates achieved at the optimal bactericidal concentration (OBC).[2]

Quinolones differ in their mechanisms of bacterial killing. These mechanisms affect their abilities to kill depending on the metabolic state of the bacteria. A quinolone that can kill bacteria regardless of an organism's metabolic state might have advantages in the bacteriological eradication of the infecting pathogen. All quinolones can kill actively dividing bacteria, although some newer fluoroquinolones can kill non-dividing cells.[3,4] The bactericidal activity of some quinolones depends on protein and RNA synthesis in the target bacterium.

In this study, we determined the killing mechanisms of gatifloxacin against strains of staphylococci, pneumococci and *Escherichia coli.* Ciprofloxacin, trovafloxacin and norfloxacin were included as comparators, since they represent quinolones with different bacterial killing mechanisms.[2–4]

Materials and methods

Antimicrobial agents

Gatifloxacin was obtained from Kyorin Pharmaceutical Co., Ltd, Tochigi, Japan; ciprofloxacin was prepared at Bristol-Myers Squibb France, Montpellier, France; trovafloxacin was obtained from Pfizer Inc., New York, NY, USA; and norfloxacin, chloramphenicol and rifampicin were purchased from Sigma Chemical Co., St Louis, MO, USA.

Bacterial strains

Seven clinical isolates of *E. coli* (two strains), *Streptococcus pneumoniae* (three strains) and methicillin-resistant *Staphylococcus aureus* (MRSA; two strains), collected from diverse geographical areas, were tested. In an attempt to study strains that might represent the bacterial species, strains selected for testing had quinolone MICs close to the modal quinolone MICs for the respective species.

MIC determination

MICs were determined by the agar dilution method as recommended by the NCCLS,[5] using bacterial inocula of *c.* 1.5×10^4 cfu/spot. The MIC was defined as the lowest concentration of antimicrobial agent that prevented visible growth.

OBC determination

The OBC was established by determining the viable counts at 0 and 6 h post-exposure of the bacteria to various concentrations of the quinolone. The OBC is the drug concentration yielding maximum reduction in viable cell counts.

Time–kill analysis

Time–kill analysis was performed in Mueller–Hinton broth (supplemented with 7% lysed horse blood for *Strepto-*

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coccus pneumoniae) using starting bacterial inocula of 10^5–10^6 cfu/mL. Cells were grown to logarithmic phase with 1 h pre-incubation in fresh broth before the addition of quinolone. The initial killing rate was determined by removing aliquots of the culture at 0, 1, 2 and 3 h post-inoculation with *E. coli*, and in addition, at 4 h for staphylococci, and at 4, 5 and 6 h for pneumococci. The duration of sampling for viability determination reflects the bacterial species-dependent rate of killing by quinolones.[1] Cell counts determined on culture samples taken immediately before and after quinolone addition were similar, suggesting that any drug carryover had minimal effect on viable counts. All plates for colony counts were incubated at 35°C for up to 48 h before any were considered to have no growth. The quinolone concentrations used for mechanism of killing determination were at their OBCs, and for *S. pneumoniae* A28275, at 10 × MIC. To distinguish the different killing mechanisms, the requirement for bacterial protein or RNA synthesis was determined by the addition of chloramphenicol or rifampicin (at 0.5 × MIC), respectively. The necessity of dividing cells for killing was tested by performing time–kill analysis for *E. coli* and MRSA in phosphate-buffered saline (PBS), or for pneumococci in PBS supplemented with 7% horse serum.[6]

Results and discussion

The studies of killing mechanism were carried out using quinolone concentrations equal to the OBC (Table 1). For gatifloxacin and trovafloxacin, these OBCs are achievable in humans following the standard oral dose.[7] However, the ciprofloxacin OBCs for pneumococci were higher than the C_{max} of 2.6 mg/L of ciprofloxacin following a 500 mg oral dose.[7] For the most part, the OBCs were generally 8–10 × MIC of quinolone.

The influence of chloramphenicol or rifampicin addition on quinolone bactericidal rates is summarized in Table 2.

Table 1. Quinolone MICs and OBCs for test strains

Bacterial strain	Quinolone	MIC (mg/L)	OBC (mg/L)	OBC/MIC
MRSA				
A27217	gatifloxacin	0.13	1	8
	ciprofloxacin	0.5	2	4
	trovafloxacin	0.016	0.25	16
	norfloxacin	1	4	4
A27218	gatifloxacin	0.13	1	8
	ciprofloxacin	0.5	4	8
	trovafloxacin	0.016	1	64
	norfloxacin	2	16	8
S. pneumoniae				
A28275	gatifloxacin	0.5	(5)[a]	
	ciprofloxacin	1	(10)	
	trovafloxacin	0.06	(0.6)	
A9585	gatifloxacin	0.5	4	8
	ciprofloxacin	1	8	8
	trovafloxacin	0.13	2	16
A28212	gatifloxacin	0.25	2	8
	ciprofloxacin	0.25	8	32
	trovafloxacin	0.06	1	16
E. coli				
A21766	gatifloxacin	0.016	0.06	4
	ciprofloxacin	0.008	0.06	8
	trovafloxacin	0.016	0.06	4
	norfloxacin	0.06	0.5	8
A20697	gatifloxacin	0.016	0.13	8
	ciprofloxacin	0.008	0.03	4
	trovafloxacin	0.008	0.06	8
	norfloxacin	0.06	0.25	4

[a]OBCs for *S. pneumoniae* A28275 were not determined; quinolone concentrations used in time–kill studies were at 10 × MIC.

Table 2. Quinolone killing profile for test strains

Bacterial strain (initial inoculum)	Quinolone	Viability (cfu/mL)[a] quinolone alone	quinolone + chloramphenicol	quinolone + rifampicin	saline	Need for protein–RNA synthesis/ dividing cells[b]
MRSA						
A27217	gatifloxacin	1×10^2	8×10^2	4×10^2	9×10^2	no/no
(9×10^5)	ciprofloxacin	8×10^2	9×10^4	5×10^3	9×10^2	yes/no
	trovafloxacin	6×10^2	6×10^3	8×10^3	8×10^4	yes/yes
	norfloxacin	6×10^2	5×10^5	5×10^4	9×10^2	yes/no
	(saline)				(8×10^4)[c]	
A27218	gatifloxacin	2×10^3	2×10^3	2×10^3	7×10^2	no/no
(8×10^5)	ciprofloxacin	8×10^3	2×10^4	5×10^3	4×10^2	no/no
	trovafloxacin	7×10^2	1×10^3	9×10^2	3×10^4	no/yes
	norfloxacin	7×10^3	3×10^4	5×10^3	3×10^2	no/no
	(saline)				(1×10^5)	
S. pneumoniae						
A28275	gatifloxacin	4×10^3	9×10^3	2×10^3	ND[d]	no/ND
(7×10^5)	ciprofloxacin	4×10^3	1×10^4	2×10^4		no/ND
	trovafloxacin	2×10^3	1×10^4	1×10^4		no/ND
A9585	gatifloxacin	8×10^4	2×10^5	2×10^5	ND	no/ND
(6×10^6)	ciprofloxacin	4×10^5	4×10^5	8×10^5		no/ND
	trovafloxacin	8×10^4	2×10^5	2×10^5		no/ND
A28212	gatifloxacin	6×10^4	6×10^4	5×10^4	ND	no/ND
(2×10^6)	ciprofloxacin	1×10^5	5×10^4	7×10^4		no/ND
	trovafloxacin	3.6×10^4	4×10^4	4×10^4		no/ND
E. coli						
A21766	gatifloxacin	$<2 \times 10^1$	1×10^2	$<2 \times 10^1$	2×10^4	no/no
(9×10^5)	ciprofloxacin	$<2 \times 10^1$	8×10^2	8×10^1	7×10^4	yes/yes
	trovafloxacin	4×10^1	6×10^3	4×10^1	8×10^3	yes/no
	norfloxacin	8×10^1	9×10^2	8×10^1	8×10^5	yes/yes
	(saline)				(7×10^5)	
A20697	gatifloxacin	$<2 \times 10^1$	$<2 \times 10^1$	$<2 \times 10^1$	5×10^3	no/no
(6×10^5)	ciprofloxacin	$<2 \times 10^1$	8×10^2	$<2 \times 10^1$	3×10^4	yes/yes
	trovafloxacin	$<2 \times 10^1$	5×10^2	$<2 \times 10^1$	8×10^4	yes/yes
	norfloxacin	1×10^2	9×10^2	1×10^2	9×10^4	no/yes
	(saline)				(1×10^5)	

[a]cfu/mL, as determined at 4 h post-inoculation for MRSA, 6 h for pneumococci and 3 h for *E. coli*, in the presence of quinolone alone or with chloramphenicol or rifampicin. Viability was determined from cultures grown in Mueller–Hinton broth (MRSA and *E. coli*), Mueller–Hinton broth + 7% lysed horse blood (pneumococci), or from bacterial suspensions in PBS.

[b]Quinolone killing reduced by the addition of protein or RNA synthesis inhibitor: cfu/mL increased by $\geq 1 \log_{10}$ (yes) or $<1 \log_{10}$ (no). Quinolone killing in PBS, as compared with cell count in saline: cfu/mL decreased by $\geq 1.5 \log_{10}$ (no) or $<1.5 \log_{10}$ (yes).

[c]cfu/mL value in parentheses is the cell count at 4 h post-inoculation of MRSA in PBS and at 3 h for *E. coli.*

[d]ND, not determinable owing to autolysis of pneumococci in PBS + 7% horse serum.

For the staphylococcal, pneumococcal and *E. coli* strains examined, gatifloxacin killing did not require protein or RNA synthesis. In contrast, ciprofloxacin, trovafloxacin and norfloxacin killing was reduced by bacterial protein or RNA synthesis inhibitors against three of the seven strains tested (Table 2). We reported previously that the presence of protein synthesis inhibitors or rifampicin may influence the bactericidal activity of gatifloxacin and ciprofloxacin against enterococci.[8]

The ability of quinolones to kill non-dividing cells is reviewed in Table 2. Killing assessment of quinolones against non-growing pneumococci was not possible due to autolysis of this organism in PBS, even with 7% horse serum supplementation. Gatifloxacin can kill non-growing staphylococcal and *E. coli* cells. Trovafloxacin killing was not observed in three strains when the staphylococci or *E. coli* A20697 were suspended in PBS.

Quinolone killing rates are organism group dependent.

Quinolones kill enteric bacilli more rapidly than staphylococci, which in turn are killed more rapidly than streptococci and enterococci.[1] Moreover, quinolones reportedly differ in their killing mechanisms depending on the organism group. For example, ciprofloxacin reportedly kills both dividing and non-dividing *E. coli* cells, but only dividing staphylococcal cells.[9,10] In this study, however, ciprofloxacin was able to kill the two staphylococcal strains under non-growing conditions, but not the *E. coli* cells suspended in saline (Table 2). Differences in testing media and other experimental differences, such as use of exponentially growing cells versus overnight cultures for the bacterial inoculum, could account for differences in the killing profile. Nonetheless, quinolone killing may differ for strains within the same organism group, as with trovafloxacin killing of the two *E. coli* strains.

In summary, relative to the comparative quinolones assessed, gatifloxacin exhibited a more favourable bactericidal profile. Gatifloxacin killed staphylococci and *E. coli* regardless of the metabolic state of the microbe. It also kills pneumococci in the absence of protein and RNA synthesis.

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