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Pharmacodynamic Properties of BAY 12-8039 on Gram-Positive and Gram-Negative Organisms as Demonstrated by Studies of Time-Kill Kinetics and Postantibiotic Effect

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Time-kill kinetics of BAY 12-8039 were studied at two inocula against three strains each of *Bacteroides fragilis*, *Escherichia coli*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Streptococcus pyogenes*. The postantibiotic effects of BAY 12-8039 were studied on three strains each of *E. coli*, *S. aureus*, *H. influenzae*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. The pharmacodynamic data demonstrated that BAY 12-8039 has marked activity against gram-positive and gram-negative organisms (under both anaerobic and aerobic conditions) and anaerobes. BAY 12-8039 also exhibited a postantibiotic effect of >1 h for all strains except one *E. coli* strain.

BAY 12-8039 is a novel fluoroquinolone with activity against gram-positive and gram-negative bacteria but not *Pseudomonas aeruginosa* (4). It has been suggested that the bactericidal activities of fluoroquinolones require the presence of oxygen (9), although reports of the activities of quinolones under anaerobic conditions are conflicting. The pharmacodynamics of an antibiotic may be investigated in several ways, including the study of time-kill kinetics and postantibiotic effect (PAE). Time-kill curves are pharmacodynamic examples of bactericidal activity expressed as the rate of killing by a fixed concentration of an antimicrobial and are one of the most reliable methods of determining tolerance (11). PAE describes the continued suppression of an organism's growth after a short exposure to an antimicrobial agent (3). We investigated the rates of killing and PAEs of BAY 12-8039 with organisms that commonly cause respiratory, urinary, skin, and soft tissue infections.

MICs were determined by broth dilution (12) with an inoculum of approximately 5×10^5 CFU/ml. MICs were determined and a time-kill kinetic study of the anaerobes and an anaerobic activity comparison were performed in an anaerobic cabinet (Don Whitley Scientific Ltd., Skipton, United Kingdom) containing an atmosphere of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen. Wilkins-Chalgren agar and broth (Unipath, Basingstoke, United Kingdom) were used for the anaerobes. Iso-Sensitest agar and broth (Unipath) were employed for the culture of aerobes and for aerobic and anaerobic activity comparisons. For *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*, these were supplemented with 20 mg of NAD (Sigma, Poole, United Kingdom) per liter and 5% horse blood (Bradsure Biologicals, Leicester, United Kingdom).

Three recent clinical isolates of *Streptococcus pyogenes* and two recent clinical isolates and the type culture (in parentheses) of each of the following organisms were studied for the time-kill kinetic investigations: *Bacteroides fragilis* (NCTC 9343), *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (ATCC 29213), and *Haemophilus influenzae* (NCTC 10479). The type culture strains of *E. coli* and *S. aureus* were used to compare the activity of BAY 12-8039 with that of cefoxitin both anaerobically and aerobically.

BAY 12-8039 (Bayer AG, Wuppertal, Germany), cefoxitin (Merck Sharpe & Dohme, Hoddesdon, United Kingdom), and metronidazole (Rhone-Poulenc Rorer, Dagenham, United Kingdom) solutions were sterilized through a 0.2 μm-pore-size filter (Sartorius AG, Gottingen, Germany) and added to logarithmic-phase broth cultures of approximately 10^5 and 10^7 CFU/ml to give concentrations equivalent to 2 and 10 times the MIC. Viable counts were determined at 0, 2, 4, 6, and 24 h after the addition of antibiotic by the Miles and Misra (8) technique on Iso-Sensitest agar plates following serial dilution in phosphate-buffered saline (pH 7.3; Unipath), and bacteria were enumerated after a 48-h incubation at 35 to 37°C. Bactericidal activity was defined as a 3 $\log_{10}$ decrease (99.9% kill) in CFU per milliliter, and bacteriostatic activity was defined as a <2.0 $\log_{10}$ decrease in CFU per milliliter.

Three recent clinical isolates of *Streptococcus pyogenes* and two recent clinical isolates and a control strain (in parentheses) of each of the following organisms were studied for the PAE investigations: *E. coli* (NCTC 10418), *S. aureus* (ATCC 29213), *H. influenzae* (NCTC 10479), and *Streptococcus pneumoniae* (NCTC 7465). The PAEs of BAY 12-8039 were determined in Iso-Sensitest broth (supplemented as described above) at a range of concentrations equivalent to 1, 4, and 10 times the MIC. Filter-sterilized BAY 12-8039 solutions were added to logarithmic-phase broth cultures of approximately 10^5 CFU/ml; a non-BAY 12-8039-exposed culture was included as a growth control. The antibiotic concentration was reduced after 1 h by a 1,000-fold dilution in prewarmed Iso-Sensitest broth (supplemented as necessary) and incubated at 35 to 37°C for 24 h. Viable counts were determined on antibiotic-free Iso-Sensitest agar plates (supplemented as necessary) prior to exposure and hourly for 6 h and at 24 h after neutralization by dilution. Viable counts were determined with a spiral plater (Don Whitley Scientific Ltd.) following dilution in phosphate-buffered saline, pH 7.3. Bacteria were enumerated after a 24-h incubation at 35 to 37°C. PAE was defined as $T - C$, where T is the time required for the count in the test culture to increase 1 $\log_{10}$ above the count observed immediately after dilution and C is the time required for the count in the untreated

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TABLE 1. MICs of BAY 12-8039

Strain	BAY 12-8039 MIC (mg/liter)
B. fragilis NCTC 9343	0.25
B. fragilis B1	0.25
B. fragilis B2	0.25
E. coli NCTC 10418	0.06
E. coli I537	0.12
E. coli I541	0.12
S. aureus ATCC 29213	0.12
S. aureus F104	0.12
S. aureus F551	0.12
H. influenzae NCTC 10479	0.03
H. influenzae A40	0.03
H. influenzae A330	0.03
Streptococcus pyogenes P44	0.5
Streptococcus pyogenes P199	0.25
Streptococcus pyogenes P404	0.25
Streptococcus pneumoniae NCTC 7465	0.25
Streptococcus pneumoniae P591	0.25
Streptococcus pneumoniae P416	0.25

TABLE 2. BAY 12-8039 PAEs

Strain	PAE (h)		
	1 × MIC	4 × MIC	10 × MIC
E. coli NCTC 10418	0.4	0.7	0.3
E. coli I537	1.0	2.2	3.2
E. coli I541	1.1	1.6	2.1
S. aureus ATCC 29213	0.9	1.4	2.1
S. aureus F551	0.9	1.8	3.3
S. aureus F104	1.8	2.7	2.8
H. influenzae NCTC 10479	0.0	1.2	3.1
H. influenzae A40	0.5	2.0	1.4
H. influenzae A330	0.7	3.1	2.0
Streptococcus pneumoniae NCTC 7465	0.3	1.2	2.0
Streptococcus pneumoniae P591	0.6	2.2	2.7
Streptococcus pneumoniae P416	1.2	1.4	2.9
Streptococcus pyogenes P44	1.6	ND	2.6
Streptococcus pyogenes P199	0.3	1.3	2.6
Streptococcus pyogenes P404	2.2	3.0	3.3

control to increase 1 $\log_{10}$ above the count observed immediately after dilution (3).

The MICs (Table 1) confirm that BAY 12-8039 has activity against gram-positive organisms, gram-negative organisms, and anaerobes (4). MICs for *E. coli* (NCTC 10418) and *S. aureus* (ATCC 29213) were the same or within one dilution step under both anaerobic and aerobic conditions. Figure 1 graphically depicts the time-kill curves for BAY 12-8039 for one strain of each species studied at an inoculum of approximately 10^5 CFU/ml. BAY 12-8039 demonstrated a bactericidal effect with all strains studied, exhibiting the least rapid bactericidal effect with *Streptococcus pyogenes* and the most rapid effects with *S. aureus* and *E. coli*. BAY 12-8039 and metronidazole exhibited similar activities against one strain of *B. fragilis*. BAY 12-8039 demonstrated a bactericidal effect at <2 h at all three concentrations and under both aerobic and anaerobic conditions with *E. coli* and at <2 h at concentrations of 4 and 10 times the MIC under both conditions with *S. aureus*. BAY 12-8039 was considerably more bactericidal than cefoxitin. Table 2 summarizes the PAEs (in hours) of BAY 12-8039

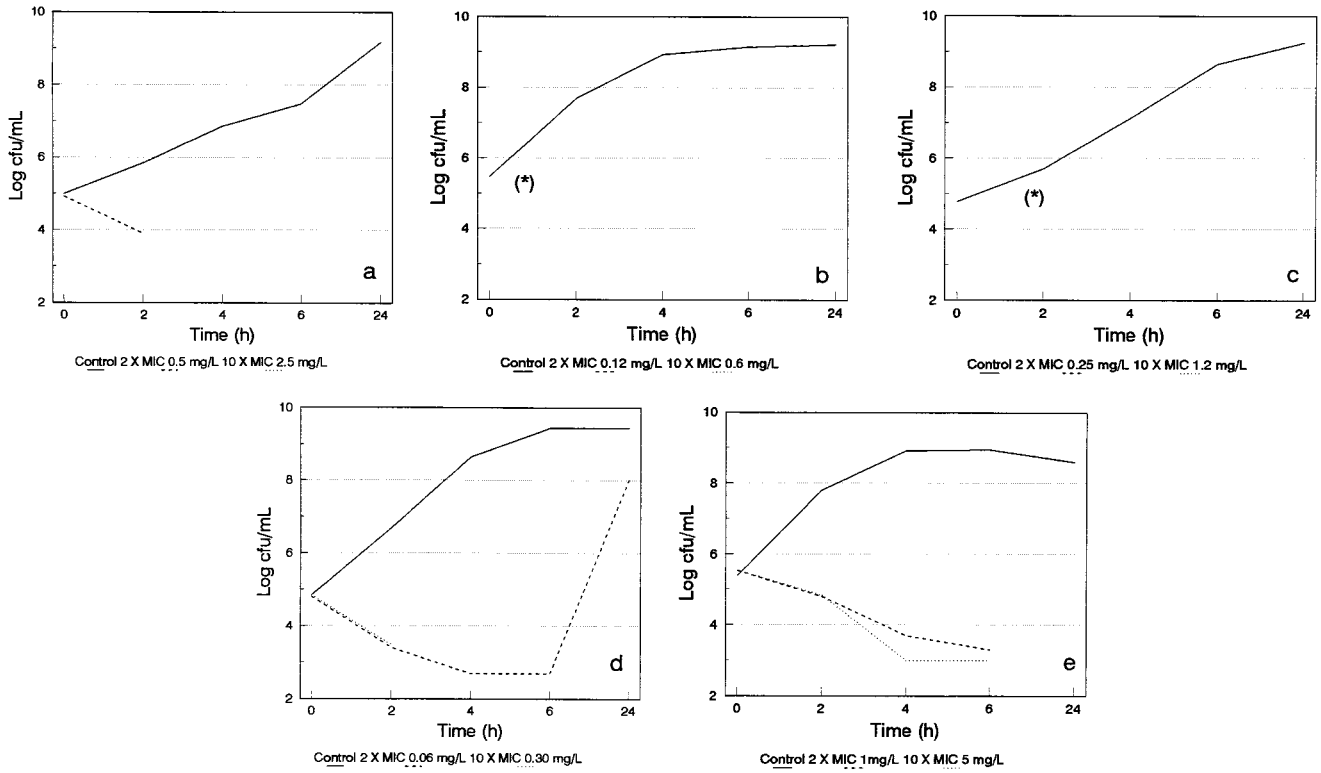

FIG. 1. BAY 12-8039 time-kill curves for *B. fragilis* B1 (a), *E. coli* I537 (b), *S. aureus* F551 (c), *H. influenzae* A40 (d), and *Streptococcus pyogenes* P44 (e). Where data are not shown (*), the $\log_{10}$ CFU/milliliter over 2 to 24 h was <2.7.

for the strains investigated. A significant PAE (>1 h) was observed with all strains except *E. coli* NCTC 10418.

The time-kill kinetic data demonstrate that BAY 12-8039 has activity against both gram-positive and gram-negative organisms (under both aerobic and anaerobic conditions) and against anaerobes. BAY 12-8039 exhibits concentration-dependent bactericidal activity and a negligible inoculum effect. Reports of quinolone activity under anaerobic conditions are conflicting. Investigators have stated that oxygen appears to be required for the bactericidal activities of some quinolones, namely ciprofloxacin and ofloxacin (9) and sparfloxacin (7). Conversely, it has been stated that the following are bactericidal in the absence of oxygen: PD127,391-2 (10), sparfloxacin and ciprofloxacin (2), fleroxacin (5), trovafloxacin (1), and ciprofloxacin, ofloxacin, temafloxacin, sparfloxacin, and clinafloxacin (13). The PAE of BAY 12-8039 is similar to those of other fluoroquinolones and increases with increasing concentration. An association between the bactericidal activity of BAY 12-8039 and the duration of its PAE was not observed.

In conclusion, BAY 12-8039 exhibits the pharmacodynamic properties expected for a fluoroquinolone, that is, bactericidal activity and a significant PAE. In addition, BAY 12-8039, unlike ciprofloxacin, exhibits concentration-dependent killing on both gram-negative and gram-positive organisms (6), although confirmation by direct experimental comparison is necessary. The clinical implications from these studies are that BAY 12-8039 is worthy of study as a single agent in the therapy of mixed anaerobic and aerobic pathogens.

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