ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1997, p. 847–849 Vol. 41, No. 4
0066-4804/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Bactericidal Activity of DU-6859a Compared to Activities of Three Quinolones, Three β-Lactams, Clindamycin, and Metronidazole against Anaerobes as Determined by Time-Kill Methodology

SHEILA K. SPANGLER,[1] MICHAEL R. JACOBS,[2] AND PETER C. APPELBAUM[1]*

Departments of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Case Western Reserve University, Cleveland, Ohio 44106[2]

Received 27 September 1996/Returned for modification 16 December 1996/Accepted 13 January 1997

The activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, piperacillin, piperacillin-tazobactam, imipenem, clindamycin, and metronidazole against 11 anaerobes were tested by the broth microdilution and time-kill methods. DU-6859a was the most active drug tested (broth microdilution MICs, 0.06 to 0.5 μg/ml), followed by imipenem (MICs, 0.002 to 4.0 μg/ml). Broth macrodilution MICs were within 3 (but usually 1) dilutions of the broth microdilution MICs. All compounds were bactericidal at the MIC after 48 h; after 24 h, 90% killing was shown for all strains when the compounds were used at four times the MIC. DU-6859a at ≤0.5 μg/ml was bactericidal after 48 h.

Most available and experimental quinolones are inactive or are only marginally active against anaerobes (1). Experimental quinolones and naphthyridones with improved antianaerobic activities include clinafloxacin, DU-6859a, and trovafloxacin (1). DU-6859a is a new broad-spectrum quinolone active against gram-positive and -negative bacteria (3, 5–8, 11, 15), including anaerobes (4, 16). We have used a time-kill method developed in our laboratory (13) to test the activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, piperacillin, piperacillin-tazobactam, imipenem, clindamycin, and metronidazole against 11 anaerobes.

The 11 strains tested (Table 1) were recent clinical isolates chosen to represent commonly occurring species from as many clinically relevant groups as possible and were identified by standard procedures (14). Broth microdilution susceptibility testing was performed by the standard methodology (9). Tazobactam was added to piperacillin at a fixed inhibitor concentration of 4.0 μg/ml. Trays were incubated in a chamber (Coy Laboratory Products, Ann Arbor, Mich.) for 48 h. β-Lactamase determination was by the nitrocefin disk method (2).

Inocula for time-kill studies were prepared inside the chamber. Five colonies from plates were suspended in 4 ml of prereduced brucella broth (Difco Laboratories) and the suspension was vortexed. A 100-μl aliquot of this suspension was added to 3.6 ml of prereduced Wilkins-Chalgren broth (Difco Laboratories) and 400 μl of laked horse erythrocytes. For metronidazole, thorough prereduction of which is necessary, 200 μl of Oxyrase solution (Oxyrase, Inc., Mansfield, Ohio) (12) was added. Oxyrase is an enzyme that is prepared from *Escherichia coli* cell membranes and that, in the presence of formate, lactate, and succinate, binds O_2. Suspensions were placed in borosilicate screw-cap tubes (15 by 45 mm) with 13-425 screw-thread open-top screw caps and 13-mm Teflon-faced rubber septa. The bottles were removed from the chamber and were incubated for 24 h in a shaking water bath at 35°C. Final inocula of 10^6 to 10^7 CFU/ml were prepared from 200-μl aliquots.

After the preincubation described above, antibiotics were prepared to a volume of 3.6 ml as follows. Syringes containing laked horse blood, Wilkins-Chalgren broth, and antibiotics were drawn up separately inside the chamber, and appropriate volumes were mixed in screw-cap tubes with septa; the introduction of air was avoided. Ranges included 3 dilutions above and 3 dilutions below the broth microdilution MIC. One antibiotic-free growth control was used in each experiment. Aliquots containing 200 μl of appropriately diluted inoculum were added, with the final inoculum being 10^6 to 10^7 CFU/ml. Suspensions were incubated at 35°C in a shaking water bath, and viability counts were performed at 0, 6, 12, 24, and 48 h by incubating the plates for 48 h inside the chamber. Only plates yielding 30 to 300 colonies were selected. Each experiment was done in duplicate, and the mean of two almost identical results was calculated. Data were analyzed by expressing growth as the $\log_{10}$ CFU per milliliter higher or lower than the original inoculum at 0 h. Bacteriostatic activity was defined as a change of 0 to 3 $\log_{10}$ CFU/ml, and bactericidal activity was defined as a change of >3 $\log_{10}$ CFU/ml at 48 h compared to that at 0 h. Drug carryover was minimized as described previously (10).

All gram-negative rods except *Fusobacterium nucleatum* and *Fusobacterium mortiferum* were β-lactamase positive; enzyme production was not demonstrated in gram-positive strains. Broth microdilution MICs and the broth macrodilution bacteriostatic and bactericidal concentrations are listed in Table 1. DU-6859a was the most active agent tested (broth microdilution MICs, 0.06 to 0.5 μg/ml), followed by imipenem MICs, 0.002 to 4.0 μg/ml). Metronidazole was active (MICs, 0.25 to 2.0 μg/ml) against all strains except *Peptostreptococcus magnus* and *Propionibacterium acnes*. Clindamycin was active (MICs, 0.03 to 0.5 μg/ml) against all strains except *Clostridium difficile*. Piperacillin-tazobactam yielded low MICs (0.125 to 1.0 μg/ml) for all β-lactamase-negative organisms except *C. difficile* and lower MICs (0.125 to 4.0 μg/ml) than those obtained with the β-lactam alone (MICs, 4.0 to 64.0 μg/ml) for β-lactam producers.

Broth macrodilution bacteriostatic and bactericidal concentrations at 48 h were within 3 (but usually 1) dilutions of the

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelba@psuhmc.hmc.psu.edu.

TABLE 1. Broth microdilution MICs compared to broth macrodilution bacteriostatic and bactericidal concentrations at 48 h from time-kill experiments

Drug	Broth microdilution MIC (μg/ml)[a]										
	Bacteroides fragilis	*Bacteroides thetaiotaomicron*	*Prevotella bivia*	*Prevotella intermedia*	*Prevotella melaninogenica*	*Fusobacterium nucleatum*	*Fusobacterium mortiferum*	*Peptostreptococcus magnus*	*Propionibacterium acnes*	*Clostridium per fringens*	*Clostridium difficile*
DU-6859a	0.5 (0.25/0.5)	0.5 (0.5/0.5)	0.25 (0.25/0.25)	0.06 (0.03/0.06)	0.12 (0.06/0.12)	0.06 (0.06/0.12)	0.25 (0.12/0.25)	0.03 (0.015/0.03)	0.03 (0.015/0.03)	0.12 (0.12/0.12)	0.12 (0.25/0.25)
Ciprofloxacin	32 (16/32)	16 (8/16)	8 (8/8)	1 (0.5/1)	4 (4/4)	1 (0.5/1)	2 (2/2)	2 (1/2)	0.12 (0.12/0.12)	0.25 (0.25/0.25)	1 (1/1)
Levofloxacin	2 (2/4)	4 (2/4)	8 (8/8)	0.5 (0.5/1)	0.5 (0.25/0.5)	0.5 (0.5/1)	4 (4/4)	2 (1/2)	1 (0.12/0.25)	0.12 (0.12/0.12)	2 (1/1)
Sparfloxacin	2 (2/8)	2 (0.5/1)	8 (8/8)	0.5 (0.25/0.5)	4 (2/4)	0.5 (0.25/0.5)	1 (0.5/1)	1 (1/1)	0.06 (0.03/0.06)	0.06 (0.06/0.06)	0.5 (0.5/0.5)
Piperacillin	64 (64/64)	32 (16/32)	4 (8/8)	16 (8/16)	8 (8/8)	2 (1/2)	1 (1/1)	0.5 (0.25/0.5)	0.5 (0.5/0.5)	0.12 (0.12/0.12)	16 (8/8)
Piperacillin-tazobactam	4 (2/8)	4 (2/4)	0.12 (0.06/0.12)	4 (2/4)	0.25 (0.12/0.25)	0.5 (0.5/1)	1 (1/1)	0.5 (0.25/0.5)	0.5 (0.25/0.5)	0.12 (0.06/0.12)	16 (8/8)
Imipenem	0.25 (0.12/0.25)	0.12 (0.06/0.12)	0.06 (0.03/0.06)	0.03 (0.03/0.03)	0.03 (0.015/0.03)	0.25 (0.25/0.25)	0.5 (0.25/0.5)	0.03 (0.03/0.03)	0.002 (0.001/0.002)	0.12 (0.12/0.12)	4 (4/4)
Clindamycin	0.25 (0.5/1)	0.5 (0.5/1)	0.25 (0.12/0.25)	0.03 (0.015/0.03)	0.5 (0.25/0.5)	0.12 (0.12/0.25)	0.25 (0.12/0.5)	0.12 (0.06/0.12)	0.5 (0.5/0.5)	0.25 (0.25/0.25)	16 (16/16)
Metronidazole	1 (1/1)	1 (2/2)	1 (1/1)	1 (1/1)	1 (1/1)	0.25 (0.12/0.25)	0.5 (1/2)	64 (64/64)	32 (16/32)	1 (1/1)	2 (2/2)

[a] Values in parentheses are broth macrodilution bacteriostatic/bactericidal concentrations (in micrograms per milliliter).

TABLE 2. Time-kill study results after 24 h and 48 h

Drug and concn	No. of strains with the decrease in original inoculum (log_{10} CFU/ml) at the following times:					
	24 h			48 h		
	−1	−2	−3	−1	−2	−3
DU-6859a						
4× MIC	11	10	9	11	11	11
2× MIC	11	10	7	11	11	11
MIC	11	9	5	11	11	11
Ciprofloxacin						
4× MIC	11	10	7	11	11	11
2× MIC	11	9	4	11	11	11
MIC	11	8	0	11	11	11
Levofloxacin						
4× MIC	11	11	8	11	11	11
2× MIC	11	10	6	11	11	11
MIC	11	10	4	11	11	11
Sparfloxacin						
4× MIC	11	10	7	11	11	11
2× MIC	10	10	5	11	11	11
MIC	10	8	3	11	11	11
Piperacillin						
4× MIC	11	8	6	11	11	11
2× MIC	10	8	6	11	11	11
MIC	10	7	5	11	11	11
Piperacillin-tazobactam						
4× MIC	11	7	6	11	11	11
2× MIC	10	7	6	11	11	11
MIC	10	7	5	11	11	11
Imipenem						
4× MIC	11	9	7	11	11	11
2× MIC	11	8	7	11	11	11
MIC	11	8	6	11	11	11
Clindamycin						
4× MIC	11	11	8	11	11	11
2× MIC	11	9	5	11	11	11
MIC	11	3	4	11	11	11
Metronidazole						
4× MIC	11	9	4	11	11	11
2× MIC	10	7	4	11	11	11
MIC	8	6	3	11	11	11

broth microdilution MICs. Bactericidal concentrations were 1 dilution higher than bacteriostatic levels, with the exceptions of sparfloxacin and piperacillin-tazobactam for *Bacteroides fragilis* and clindamycin for *F. mortiferum*, for which bactericidal concentrations were 2 dilutions higher than bacteriostatic concentrations. DU-6859a yielded the lowest bactericidal concentrations ($\leq$0.5 μg/ml) (Table 1).

Time-kill study results after 24 and 48 h are presented in Table 2. Killing rates for all drugs against all strains were similar for these two periods. All compounds were bactericidal at the MIC after 48 h and gave 90% killing after 24 h at four times the MIC. DU-6859a killed 99% of 10 of 11 strains at two times the MIC after 24 h. Ciprofloxacin killed 90% of all strains at the MIC after 24 h. Levofloxacin at four times the MIC killed 99% of all strains, and at the MIC, levofloxacin killed 90% of all strains after 24 h. Sparfloxacin at four times the MIC killed 90% of all strains after 24 h. Piperacillin and piperacillin-tazobactam at four times the MIC killed 90% of all

strains after 24 h. Imipenem at the MIC killed 90% of all strains after 24 h. Clindamycin at four times the MIC killed 99% of all strains after 24 h, and at the MIC it killed 90% of all strains after 24 h. Metronidazole at four times the MIC killed 90% of all strains after 24 h. After 12 h, DU-6859a killed 99% (9 of 11) strains, and clindamycin at four times the MIC killed 7 of 11 strains, with lower killing rates by other compounds. After 6 h, DU-6859a, levofloxacin, and clindamycin showed more rapid 90% killing than the other compounds. No significant killing was found at sub-MICs, and no interspecies differences in kinetics were observed.

Kato and coworkers (4) and Wexler et al. (16) have reported that DU-6859a has excellent activity against anaerobes, with all strains inhibited at an MIC of ≤1.0 μg/ml and with overall MICs at which 50 and 90% of isolates are inhibited of 0.125 and 0.25 μg/ml, respectively. Our results confirm these MICs and further characterize the excellent activity of DU-6859a against anaerobes compared to those of other quinolones and nonquinolones by the time-kill methodology. DU-6859a at ≤0.5 μg/ml was found to have bactericidal activity against all 11 strains tested after 48 h.

Because of the paucity of strains tested, results are preliminary and should be confirmed by testing more species. The clinical significance of more rapid killing by DU-6859a, levofloxacin, and clindamycin at earlier intervals is unknown at present.

The results of the current study, together with previously published MIC data, point to the need for clinical trials of DU-6859a for the treatment of infections caused by anaerobes and both aerobes and anaerobes.

This study was supported by a grant from Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.

We thank J. Copeland (Mansfield, Ohio) for provision of Oxydishes and Oxyrase.

REFERENCES

1. **Appelbaum, P. C.** 1995. Quinolone activity against anaerobes: microbiological aspects. Drugs **49**(Suppl. 2)**:**76–80.
2. **Jacobs, M. R., S. K. Spangler, and P. C. Appelbaum.** 1992. Beta-lactamase production and susceptibility of US and European anaerobic gram-negative bacilli to beta-lactams and other agents. Eur. J. Clin. Microbiol. Infect. Dis. **11:**1081–1093.
3. **Jolley, A., J. M. Andrews, N. Brenwald, and R. Wise.** 1993. The in-vitro activity of a new highly active quinolone, DU-6859a. J. Antimicrob. Chemother. **32:**757–763.
4. **Kato, N., K. Watanabe, H. Kato, K. Tanaka, Y. Tanaka, and K. Ueno.** 1993. Comparative in vitro activity of DU-6859a, a new quinolone, against anaerobic bacteria, abstr. 990, p. 300. *In* Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
5. **Korten, V., J. F. Tomayko, and B. E. Murray.** 1994. Comparative in vitro activity of DU-6859a, a new fluoroquinolone agent, against gram-positive cocci. Antimicrob. Agents Chemother. **38:**611–615.
6. **Marshall, S. A., and R. N. Jones.** 1993. In vitro activity of DU-6859a, a new fluorocyclopropyl quinolone. Antimicrob. Agents Chemother. **37:**2747–2753.
7. **Marshall, S. A., R. N. Jones, P. R. Murray, J. A. Washington, S. D. Allen, E. H. Gerlach, and M. E. Erwin.** 1993. In-vitro comparison of DU-6859a, a novel fluoroquinolone, with other quinolones and oral cephalosporins tested against 5086 recent clinical isolates. J. Antimicrob. Chemother. **32:**877–884.
8. **Nakane, T., S. Iyobe, K. Sato, and S. Mitsuhashi.** 1995. In vitro antibacterial activity of DU-6859a, a new fluoroquinolone. Antimicrob. Agents Chemother. **39:**2822–2826.
9. **National Committee for Clinical Laboratory Standards.** 1993. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 3rd ed. Approved standard. NCCLS publication M11-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. **38:**2317–2322.
11. **Sato, K., K. Hoshino, M. Tanaka, I. Hayakawa, and Y. Osada.** 1992. Antimicrobial activity of DU-6859a, a new potent fluoroquinolone, against clinical isolates. Antimicrob. Agents Chemother. **36:**1491–1498.
12. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1994. Effect of CO_2 on susceptibilities of anaerobes to erythromycin, azithromycin, clarithromycin and roxithromycin. Antimicrob. Agents Chemother. **38:**211–216.
13. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** J. Antimicrob. Chemother., in press.
14. **Summanen, P., E. J. Baron, D. M. Citron, C. A. Strong, H. M. Wexler, and S. M. Finegold.** 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Company, Belmont, Calif.
15. **Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum.** 1996. MIC and time-kill study of activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, cefotaxime, imipenem, and vancomycin against nine penicillin-susceptible and -resistant pneumococci. Antimicrob. Agents Chemother. **40:**362–366.
16. **Wexler, H. M., E. Molitoris, D. Reeves, and S. M. Finegold.** 1994. In vitro activity of DU-6859a against anaerobic bacteria. Antimicrob. Agents Chemother. **38:**2504–2509.