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Antianaerobic Activity of the Ketolide RU 64004 Compared to Activities of Four Macrolides, Five β-Lactams, Clindamycin, and Metronidazole

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Agar dilution methodology (with added Oxyrase in the case of the macrolide group to allow incubation without added CO_2) was used to compare the activity of RU 64004, a new ketolide, with the activities of erythromycin, azithromycin, clarithromycin, roxithromycin, clindamycin, amoxicillin with and without clavulanate, piperacillin with and without tazobactam, metronidazole, and imipenem against 379 anaerobes. Overall, RU 64004 yielded an MIC at which 50% of the isolates are inhibited (MIC_{50}) of 1.0 μg/ml and an MIC_{90} of 16.0 μg/ml. In comparison, MIC_{50}s and MIC_{90}s of erythromycin, azithromycin, clarithromycin, and roxithromycin were 2.0 to 8.0 and >64.0 μg/ml, respectively. MICs of macrolides, including RU 64004, were higher for *Bacteroides ovatus*, *Fusobacterium varium*, *Fusobacterium mortiferum*, and *Clostridium difficile* than for the other species. RU 64004 was more active against gram-positive rods and cocci, *Prevotella* and *Porphyromonas* spp., and fusobacteria other than *F. mortiferum* and *F. varium* than against the *Bacteroides fragilis* group. Overall MIC_{50}s and MIC_{90}s (in micrograms per milliliter), respectively, of other compounds were as follows: clindamycin, 1.0 and 16.0; amoxicillin, 4.0 and 64.0; amoxicillin-clavulanate, 0.5 and 4.0; piperacillin, 8.0 and >64.0; piperacillin-tazobactam, 1.0 and 16.0; metronidazole, 1.0 and 4.0; and imipenem, 0.25 and 1.0.

Susceptibility testing of macrolides and azalides in the presence of CO_2 may lead to elevated MICs due to a CO_2-dependent decrease in the pH of the medium (5, 11, 12, 14–19). Because MICs for anaerobes are usually determined in an atmosphere of N_2, H_2, and CO_2 in an anaerobic chamber or anaerobic jars, it has generally been assumed that these compounds have low activity against this class of organism. However, Retsema and coworkers (16), Nachnani and coworkers (12), and Barry and Fuchs (4) have demonstrated by microdilution MIC testing that the absence of added CO_2 in the incubation atmosphere when azithromycin and erythromycin are tested leads to lower MICs for aerobic as well as anaerobic organisms. Recently, we developed an agar dilution method allowing incubation without added CO_2, using Oxyrase to remove O_2 from atmospheric air, and demonstrated significantly increased susceptibility of a spectrum of gram-negative and -positive anaerobes to erythromycin, azithromycin, clarithromycin, and roxithromycin. The method has also been adapted for use with the E test (17–19).

RU 64004 is a new ketolide that is active against gram-positive and -negative aerobes (1–3, 7, 9). In the present study, we compared the activity of RU 64004 to those of erythromycin, azithromycin, clarithromycin, roxithromycin, amoxicillin with and without clavulanate, piperacillin with and without tazobactam, clindamycin, metronidazole, and imipenem against 379 anaerobes, utilizing the Oxyrase method for macrolides and standard agar dilution for β-lactams, clindamycin, and metronidazole.

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MATERIALS AND METHODS

Bacterial strains. Organisms (see Table 1) were all clinical strains isolated within 4 years of the study and identified by standard methodology (20). Organisms were stored at −70°C in double-strength skim milk (Difco Laboratories, Detroit, Mich.). Prior to testing, strains were subcultured twice onto enriched brucella blood agar plates. Purity was checked throughout the study by Gram stain and colonial morphology.

Susceptibility testing. Antibiotic powders of known potency were obtained as follows: erythromycin and clarithromycin, Abbott Laboratories, North Chicago, Ill.; azithromycin, Pfizer Inc., New York, N.Y.; RU 64004 and roxithromycin, Roussel Uclaf, Paris, France; amoxicillin and clavulanate, SmithKline Beecham Laboratories, Philadelphia, Pa.; piperacillin and tazobactam, Wyeth-Ayerst Laboratories, Pearl River, N.Y.; clindamycin, The Upjohn Co., Kalamazoo, Mich.; imipenem, Merck and Co., Rahway, N.J.; metronidazole, Sigma Chemical Co., St. Louis, Mo. β-Lactamase testing was done by the Cefinase disk method (20). Two susceptibility methods were employed. (i) For all compounds except macrolides, MICs were determined by the agar dilution method recommended by the National Committee for Clinical Laboratory Standards (13) with Wilkins-Chalgren agar (supplemented with 5% sheep blood when non-*Bacteroides fragilis* group strains were tested) and incubation in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2. Clavulanate was added to amoxicillin in a 1:2 ratio, and tazobactam was added to piperacillin at a fixed concentration of 4.0 μg/ml. Because Oxyrase contains bacterial cell membranes which may bind to penicillin binding proteins, it was not used for β-lactams (6). Also, because no significant difference has been found with MICs of clindamycin and metronidazole with and without CO_2 (15, 17), the glove box method was employed for the sake of convenience. (ii) For macrolides, the Oxyrase method (17–19) was used as follows. To 20.5 ml of molten Wilkins-Chalgren agar were added 1.2 ml of antibiotic solution, 1.0 ml of sheep blood, and 2.3 ml of Oxyrase for agar solution containing Oxyrase plus substrates (Oxyrase, Inc., Mansfield, Ohio). The mixture was then poured into OxyDish plates (Oxyrase, Inc.). After inoculation of the plates with a Steers replicator, the plates were sealed with their lids and were incubated in air. All MIC plates were incubated at 37°C for 48 h. Quality control strains with both methods included *B. fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124.

RESULTS AND DISCUSSION

Results of the MIC testing are presented in Table 1. RU 64004 had an overall MIC at which 50% of the isolates are inhibited (MIC_{50}) of 1.0 μg/ml and an MIC_{90} of 16.0 μg/ml;

TABLE 1. Antianaerobic activity of antimicrobials

Organism[a] and drug	MIC (μg/ml)		
	Range	50%	90%
Bacteroides fragilis (64/65)			
RU 64004	1.0–16.0	4.0	8.0
Erythromycin	2.0–>64.0	16.0	32.0
Azithromycin	4.0–>64.0	16.0	32.0
Clarithromycin	1.0–>64.0	4.0	8.0
Roxithromycin	4.0–>64.0	32.0	>64.0
Clindamycin	≤0.06–>64.0	1.0	2.0
Amoxicillin	0.25–>128.0	32.0	>128.0
Amoxicillin-clavulanate	0.25–>32.0	0.5	2.0
Piperacillin	2.0–>64.0	16.0	>64.0
Piperacillin-tazobactam	≤0.125–16.0	1.0	4.0
Imipenem	0.06–2.0	0.25	0.5
Metronidazole	0.25–2.0	1.0	2.0
Bacteroides thetaiotaomicron (26/26)			
RU 64004	2.0–32.0	4.0	16.0
Erythromycin	4.0–>64.0	8.0	32.0
Azithromycin	4.0–>64.0	16.0	>64.0
Clarithromycin	2.0–>64.0	8.0	32.0
Roxithromycin	16.0–>64.0	>64.0	>64.0
Clindamycin	1.0–>64.0	4.0	8.0
Amoxicillin	16.0–>128.0	64.0	64.0
Amoxicillin-clavulanate	0.5–4.0	1.0	1.0
Piperacillin	4.0–>64.0	64.0	64.0
Piperacillin-tazobactam	0.5–>16.0	>16.0	>16.0
Imipenem	0.125–0.5	0.25	0.5
Metronidazole	0.25–2.0	1.0	2.0
Bacteroides ovatus (11/11)			
RU 64004	2.0–>64.0	4.0	32.0
Erythromycin	4.0–>64.0	8.0	>64.0
Azithromycin	1.0–>64.0	8.0	>64.0
Clarithromycin	2.0–>64.0	4.0	>64.0
Roxithromycin	8.0–>64.0	64.0	>64.0
Clindamycin	1.0–>64.0	2.0	64.0
Amoxicillin	16.0–>128.0	32.0	>128.0
Amoxicillin-clavulanate	0.5–2.0	0.5	2.0
Piperacillin	16.0–>64.0	32.0	>64.0
Piperacillin-tazobactam	2.0–16.0	4.0	8.0
Imipenem	0.125–2.0	0.25	0.5
Metronidazole	0.25–4.0	2.0	2.0
Bacteroides distasonis (12/28)			
RU 64004	0.25–16.0	1.0	4.0
Erythromycin	8.0–>64.0	32.0	>64.0
Azithromycin	4.0–>64.0	32.0	>64.0
Clarithromycin	0.5–>64.0	2.0	8.0
Roxithromycin	4.0–>64.0	32.0	>64.0
Clindamycin	0.25–>64.0	4.0	16.0
Amoxicillin	1.0–>128.0	4.0	>128.0
Amoxicillin-clavulanate	1.0–16.0	4.0	16.0
Piperacillin	4.0–>64.0	64.0	>64.0
Piperacillin-tazobactam	4.0–>16.0	16.0	>16.0
Imipenem	0.125–4.0	0.5	2.0
Metronidazole	≤0.5–2.0	1.0	2.0
Bacteroides vulgatus (14/14)			
RU 64004	0.25–16.0	1.0	2.0
Erythromycin	0.5–>64.0	4.0	4.0
Azithromycin	1.0–>64.0	4.0	16.0
Clarithromycin	0.5–>64.0	2.0	2.0
Roxithromycin	2.0–>64.0	8.0	8.0
Clindamycin	≤0.06–>64.0	0.25	1.0
Amoxicillin	8.0–>128.0	16.0	128.0
Amoxicillin-clavulanate	0.5–4.0	1.0	2.0
Piperacillin	8.0–>64.0	16.0	64.0
Piperacillin-tazobactam	2.0–8.0	4.0	8.0
Imipenem	0.125–1.0	0.5	0.5
Metronidazole	≤0.5–2.0	1.0	1.0
Bacteroides uniformis (11/11)			
RU 64004	1.0–32.0	2.0	8.0
Erythromycin	2.0–>64.0	4.0	8.0
Azithromycin	2.0–>64.0	4.0	16.0
Clarithromycin	1.0–>64.0	2.0	8.0
Roxithromycin	8.0–>64.0	16.0	32.0
Clindamycin	≤0.06–>64.0	0.25	2.0
Amoxicillin	8.0–>128.0	32.0	>128.0
Amoxicillin-clavulanate	0.25–8.0	0.5	2.0
Piperacillin	4.0–>64.0	16.0	>64.0
Piperacillin-tazobactam	0.25–8.0	1.0	2.0
Imipenem	0.12–1.0	0.25	0.25
Metronidazole	≤0.5–2.0	1.0	1.0
Bacteroides fragilis group (138/155)			
RU 64004	0.25–>64.0	4.0	8.0
Erythromycin	0.5–>64.0	16.0	>64.0
Azithromycin	1.0–>64.0	16.0	>64.0
Clarithromycin	0.5–>64.0	4.0	>64.0
Roxithromycin	2.0–>64.0	32.0	>64.0
Clindamycin	≤0.06–>64.0	1.0	64.0
Amoxicillin	0.25–>128.0	32.0	>128.0
Amoxicillin-clavulanate	0.25–>32.0	1.0	4.0
Piperacillin	2.0–>64.0	16.0	>64.0
Piperacillin-tazobactam	≤0.125–>16.0	4.0	>16.0
Imipenem	0.06–4.0	0.25	1.0
Metronidazole	≤0.5–4.0	1.0	2.0
Prevotella bivia (16/23)			
RU 64004	0.06–0.5	0.25	0.25
Erythromycin	1.0–16.0	2.0	4.0
Azithromycin	0.25–4.0	1.0	2.0
Clarithromycin	≤0.125–1.0	0.25	0.5
Roxithromycin	0.5–4.0	1.0	4.0
Clindamycin	≤0.06–0.5	≤0.06	≤0.06
Amoxicillin	≤0.125–64.0	2.0	32.0
Amoxicillin-clavulanate	≤0.125–2.0	0.25	0.5
Piperacillin	1.0–64.0	4.0	16.0
Piperacillin-tazobactam	≤0.125–0.12	≤0.125	≤0.125
Imipenem	≤0.016–0.06	0.03	0.06
Metronidazole	1.0–8.0	2.0	4.0
Miscellaneous strains[b] (19/25)			
RU 64004	≤0.008–0.03	≤0.008	≤0.008
Erythromycin	≤0.125–2.0	0.25	0.5
Azithromycin	≤0.125–1.0	0.25	0.5
Clarithromycin	≤0.125	≤0.125	≤0.125
Roxithromycin	≤0.125–1.0	0.25	0.5
Clindamycin	≤0.06	≤0.06	≤0.06
Amoxicillin	≤0.125–128.0	4.0	64.0
Amoxicillin-clavulanate	≤0.125–2.0	0.25	1.0
Piperacillin	≤0.125–>64.0	16.0	>64.0
Piperacillin-tazobactam	≤0.125	≤0.125	≤0.125
Imipenem	≤0.016–0.125	0.06	0.125
Metronidazole	≤0.5–2.0	1.0	2.0
Non-*Bacteroides fragilis Bacteroides*, *Prevotella*, and *Porphyromonas* (35/48)			
RU 64004	≤0.008–0.5	0.03	0.25
Erythromycin	≤0.125–0.5	0.5	4.0
Azithromycin	≤0.125–4.0	0.5	1.0
Clarithromycin	≤0.125–1.0	≤0.125	0.5
Roxithromycin	≤0.125–4.0	0.5	2.0

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TABLE 1—*Continued*

Organism[a] and drug	MIC (μg/ml)		
	Range	50%	90%
Clindamycin	≤0.06–0.5	≤0.06	≤0.06
Amoxicillin	≤0.125–128.0	4.0	64.0
Amoxicillin-clavulanate	≤0.125–2.0	0.25	1.0
Piperacillin	≤0.125–>64.0	8.0	64.0
Piperacillin-tazobactam	≤0.125	≤0.125	≤0.125
Imipenem	≤0.016–0.125	0.03	0.125
Metronidazole	≤.5–8.0	1.0	4.0
Fusobacterium nucleatum (2/10)			
RU 64004	0.25–4.0	0.5	2.0
Erythromycin	0.5–4.0	2.0	4.0
Azithromycin	0.5–8.0	0.25	0.5
Clarithromycin	0.5–8.0	1.0	4.0
Roxithromycin	1.0–32.0	16.0	16.0
Clindamycin	≤0.06–0.125	≤0.06	0.125
Amoxicillin	≤0.125–>128.0	0.5	>128.0
Amoxicillin-clavulanate	≤0.125–>32.0	0.5	1.0
Piperacillin	≤0.125–>64.0	≤0.125	>64.0
Piperacillin-tazobactam	≤0.125–0.5	≤0.125	0.25
Imipenem	0.03–0.25	0.125	0.25
Metronidazole	≤0.5	≤0.5	≤0.5
Fusobacterium necrophorum (0/11)			
RU 64004	0.5–4.0	2.0	2.0
Erythromycin	0.125–0.5	0.25	0.5
Azithromycin	0.125–0.5	0.25	0.5
Clarithromycin	1.0–8.0	2.0	4.0
Roxithromycin	2.0–32.0	8.0	16.0
Clindamycin	≤0.06	≤0.06	≤0.06
Amoxicillin	≤0.125–0.25	0.25	0.25
Amoxicillin-clavulanate	≤0.125–0.25	0.25	0.25
Piperacillin	≤0.125	≤0.125	≤0.125
Piperacillin-tazobactam	≤0.125	≤0.125	≤0.125
Imipenem	≤0.016–0.06	0.03	0.03
Metronidazole	≤0.5	≤0.5	≤0.5
Fusobacterium mortiferum (2/9)			
RU 64004	4.0–>64.0	32.0	
Erythromycin	4.0–>64.0	>64.0	
Azithromycin	4.0–>64.0	8.0	
Clarithromycin	4.0–>64.0	>64.0	
Roxithromycin	16.0–>64.0	>64.0	
Clindamycin	0.125–0.25	0.25	
Amoxicillin	4.0–>128.0	8.0	
Amoxicillin-clavulanate	4.0–>32.0	4.0	
Piperacillin	0.5–>64.0	0.5	
Piperacillin-tazobactam	0.5–>16.0	0.5	
Imipenem	1.0–2.0	1.0	
Metronidazole	≤0.5	≤0.5	
Fusobacterium varium (0/17)			
RU 64004	32.0–>64.0	>64.0	>64.0
Erythromycin	>64.0	>64.0	>64.0
Azithromycin	16.0–>64.0	>64.0	>64.0
Clarithromycin	>64.0	>64.0	>64.0
Roxithromycin	>64.0	>64.0	>64.0
Clindamycin	4.0–64.0	8.0	32.0
Amoxicillin	2.0–8.0	2.0	4.0
Amoxicillin-clavulanate	1.0–4.0	2.0	4.0
Piperacillin	2.0–32.0	8.0	32.0
Piperacillin-tazobactam	2.0–>16.0	8.0	8.0
Imipenem	0.5–1.0	0.5	1.0
Metronidazole	≤0.5	≤0.5	≤0.5
All fusobacteria (4/47)			
RU 64004	0.25–>64.0	32.0	>64.0
Erythromycin	0.5–>64.0	>64.0	>64.0
Azithromycin	≤0.125–>64.0	4.0	>64.0
Clarithromycin	0.5–>64.0	64.0	>64.0
Roxithromycin	1.0–>64.0	64.0	>64.0
Clindamycin	≤0.06–64.0	0.125	32.0
Amoxicillin	≤0.125–>128.0	2.0	16.0
Amoxicillin-clavulanate	≤0.125–>32.0	1.0	4.0
Piperacillin	≤0.125–>64.0	1.0	32.0
Piperacillin-tazobactam	0.25–>16.0	0.5	8.0
Imipenem	≤0.016–2.0	0.5	1.0
Metronidazole	≤0.5	≤0.5	≤0.5
Peptostreptococci[c] (0/47)			
RU 64004	≤0.008–4.0	≤0.008	0.06
Erythromycin	≤0.125–>64.0	2.0	4.0
Azithromycin	≤0.125–>64.0	2.0	64.0
Clarithromycin	≤0.125–>64.0	1.0	4.0
Roxithromycin	≤0.125–>64.0	4.0	32.0
Clindamycin	≤0.06–>64.0	0.25	2.0
Amoxicillin	≤0.125–32.0	0.25	0.5
Amoxicillin-clavulanate	≤0.125–32.0	0.25	0.25
Piperacillin	≤0.125–16.0	≤0.125	0.5
Piperacillin-tazobactam	≤0.125–16.0	≤0.125	0.5
Imipenem	≤0.016–1.0	0.06	0.25
Metronidazole	≤0.5–2.0	1.0	2.0
Propionibacteria[d] (0/12)			
RU 64004	≤0.008	≤0.008	≤0.008
Erythromycin	≤0.125–0.25	≤0.125	≤0.125
Azithromycin	≤0.125–0.25	≤0.125	≤0.125
Clarithromycin	≤0.125	≤0.125	≤0.125
Roxithromycin	≤0.125–0.25	≤0.125	≤0.125
Clindamycin	≤0.06–0.5	0.125	0.25
Amoxicillin	≤0.125–0.5	≤0.125	0.25
Amoxicillin-clavulanate	≤0.125–0.5	≤0.125	0.25
Piperacillin	0.25–2.0	0.5	2.0
Piperacillin-tazobactam	≤0.125–2.0	0.25	1.0
Imipenem	≤0.016–0.06	≤0.016	0.03
Metronidazole	>16.0	>16.0	>16.0
Other gram-positive anaerobic non-spore-forming rods[e] (0/8)			
RU 64004	≤0.008	≤0.008	
Erythromycin	≤0.125–0.25	≤0.125	
Azithromycin	≤0.125–0.5	0.25	
Clarithromycin	≤0.125–0.25	≤0.125	
Roxithromycin	≤0.125–0.5	≤0.125	
Clindamycin	≤0.06–8.0	1.0	
Amoxicillin	≤0.125–2.0	0.5	
Amoxicillin-clavulanate	≤0.125–2.0	0.5	
Piperacillin	0.25–2.0	1.0	
Piperacillin-tazobactam	0.25–2.0	1.0	
Imipenem	0.06–4.0	0.25	
Metronidazole	>16.0	>16.0	
Clostridium perfringens (0/21)			
RU 64004	≤0.008–0.06	0.03	0.03
Erythromycin	0.25–2.0	1.0	2.0
Azithromycin	0.25–1.0	0.5	1.0
Clarithromycin	0.25–1.0	0.5	1.0
Roxithromycin	1.0–4.0	2.0	2.0
Clindamycin	≤0.06–8.0	1.0	4.0
Amoxicillin	≤0.125–0.5	0.25	0.5
Amoxicillin-clavulanate	≤0.125–0.5	≤0.125	0.25
Piperacillin	≤0.125–2.0	0.5	2.0
Piperacillin-tazobactam	≤0.125–2.0	0.5	2.0
Imipenem	0.03–0.25	0.06	0.125
Metronidazole	≤0.5–4.0	≤0.5	2.0

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TABLE 1—*Continued*

Organism[a] and drug	MIC (μg/ml)		
	Range	50%	90%
Clostridium difficile (0/11)			
RU 64004	0.06–>64.0	0.12	>64.0
Erythromycin	0.5–>64.0	1.0	>64.0
Azithromycin	1.0–>64.0	1.0	>64.0
Clarithromycin	0.25–>64.0	0.5	>64.0
Roxithromycin	1.0–>64.0	1.0	>64.0
Clindamycin	8.0–>64.0	8.0	>64.0
Amoxicillin	0.5–4.0	1.0	2.0
Amoxicillin-clavulanate	0.5–2.0	0.5	1.0
Piperacillin	2.0–8.0	8.0	8.0
Piperacillin-tazobactam	2.0–8.0	8.0	8.0
Imipenem	4.0–>8.0	4.0	>8.0
Metronidazole	≤0.5–1.0	≤0.5	≤0.5
Other clostridia[f] (0/30)			
RU 64004	≤0.008–16.0	0.016	0.03
Erythromycin	≤0.125–>64.0	0.5	2.0
Azithromycin	≤0.125–>64.0	1.0	1.0
Clarithromycin	≤0.125–>64.0	≤0.125	1.0
Roxithromycin	≤0.125–>64.0	1.0	2.0
Clindamycin	≤0.06–>64.0	2.0	16.0
Amoxicillin	≤0.125–8.0	0.25	1.0
Amoxicillin-clavulanate	≤0.125–1.0	0.25	1.0
Piperacillin	≤0.125–64.0	1.0	32.0
Piperacillin-tazobactam	≤0.125–16.0	0.5	16.0
Imipenem	≤0.016–0.5	0.125	0.5
Metronidazole	≤0.5–>16.0	≤0.5	8.0
All strains (177/379)			
RU 64004	≤0.008–>64.0	1.0	16.0
Erythromycin	≤0.125–>64.0	4.0	>64.0
Azithromycin	≤0.125–>64.0	4.0	>64.0
Clarithromycin	≤0.125–>64.0	2.0	>64.0
Roxithromycin	≤0.125–>64.0	8.0	>64.0
Clindamycin	≤0.06–>64.0	1.0	16.0
Amoxicillin	≤0.125–>128.0	4.0	64.0
Amoxicillin-clavulanate	≤0.125–>32.0	0.5	4.0
Piperacillin	≤0.125–>64.0	8.0	>64.0
Piperacillin-tazobactam	≤0.125–>16.0	1.0	16.0
Imipenem	≤0.016–>8.0	0.25	1.0
Metronidazole	≤0.5–>16.0	1.0	4.0

[a] Numbers in parentheses are number of β-lactamase-positive strains/number of strains tested.

[b] *Prevotella disiens* (8 strains), *Prevotella oralis* (3 strains), *Prevotella buccae* (1 strain), *Prevotella melaninogenica* (2 strains), *Prevotella intermedia* (9 strains), and *Porphyromonas asaccharolytica* (2 strains).

[c] *Peptostreptococcus asaccharolyticus* (13 strains), *Peptostreptococcus magnus* (16 strains), *Peptostreptococcus anaerobius* (10 strains), and *Peptostreptococcus tetradius* (8 strains).

[d] *Propionibacterium acnes* (11 strains) and *Propionibacterium* spp. (1 strain).

[e] *Actinomyces israelii* (1 strain), *Actinomyces meyeri* (1 strain), *Actinomyces naeslundii* (1 strain), and *Lactobacillus* spp. (5 strains).

[f] *Clostridium ramosum* (1 strain), *Clostridium tertium* (9 strains), *Clostridium bifermentans* (3 strains), *Clostridium sordellii* (6 strains), *Clostridium septicum* (2 strains), *Clostridium histolyticum* (1 strain), *Clostridium innocuum* (1 strain), *Clostridium cadaveris* (1 strain), and *Clostridium* spp. (6 strains).

these were similar to those of clindamycin. In comparison, the MIC_{50}s and MIC_{90}s of erythromycin, azithromycin, clarithromycin, and roxithromycin were 2.0 to 8.0 and >64.0 μg/ml, respectively.

RU 64004 was more active against the non-*B. fragilis* group anaerobic gram-negative rods (other than *Fusobacterium varium* and *Fusobacterium mortiferum*) than against the *B. fragilis* group and was very active against gram-positive anaerobes (with the exception of some strains of *Clostridium difficile*). Overall MIC_{50}s and MIC_{90}s (in micrograms per milliliter) for other compounds can be seen in Table 1. Clindamycin resistance (≥4.0 μg/ml) occurred in 62 strains (34 *B. fragilis* group strains and 28 *F. varium* strains, peptostreptococci, and clostridia). For the 34 clindamycin-resistant *B. fragilis* strains (18 *B. thetaiotaomicron* strains, 3 *Bacteroides ovatus* strains, and 13 *Bacteroides distasonis* strains), MIC_{50}s and MIC_{90}s of both RU 64004 and clindamycin were 4.0 and 8.0 μg/ml. For all 62 strains, MIC_{50}s and MIC_{90}s of RU 64004 were 2.0 and >64.0 μg/ml, respectively, and those of clindamycin were both 8.0 μg/ml. In tests with peptostreptococci, MIC_{90}s of erythromycin and clarithromycin were only two- and fourfold higher than MIC_{50}s, respectively, whereas there was a 32-fold difference in these azithromycin MICs.

RU 64004 is a new semisynthetic ketolide antimicrobial characterized by a 3-keto function which replaces the cladinose moiety of other members of the macrolide group. RU 64004 is active against staphylococci, streptococci (including *Streptococcus pneumoniae* and enterococci), several species of *Enterobacteriaceae* (with the exception of *Salmonella*), *Haemophilus influenzae*, *Moraxella catarrhalis*, *Neisseria* spp., *Legionella* spp., *Helicobacter pylori*, *Chlamydia* spp., mycoplasmas, and nontuberculous mycobacteria (1–3, 7, 9). This study indicates that RU 64004 also possesses significant antianaerobic activity.

The only anaerobe species for which RU 64004 MIC_{90}s were ≥32.0 μg/ml were *B. ovatus*, *F. varium*, and *C. difficile*; the MICs of all other members of the macrolide group were also higher for these three strains. The MIC_{50}s of all macrolides were ≥8.0 μg/ml for *F. mortiferum*. *B. ovatus*, *F. mortiferum*, and *F. varium* are infrequent human pathogens, and toxigenic strains of *C. difficile* produce a clearly defined clinical syndrome which is treated with oral vancomycin or metronidazole. The latter three species have previously been shown to be resistant to all macrolides with and without incubation in CO_2 (8, 10, 18). Compared with RU 64004, clarithromycin was the next most effective macrolide against all anaerobe strains tested, followed by erythromycin, azithromycin, and roxithromycin. However, the lowest MICs for fusobacteria were those of azithromycin. The relative antianaerobic activity of the latter three compounds has been confirmed in a previous report (18). Firm conclusions as to the relative in vitro antianaerobic activity of macrolides tested in the current study cannot be made until more studies are performed. The reason for the 32-fold difference between the MIC_{50}s and MIC_{90}s of azithromycin for peptostreptococci is unclear and could be explained by an azithromycin-specific resistance mechanism. This postulate requires investigation.

No macrolide breakpoints are currently available for anaerobes, and conclusions regarding susceptibility rates can therefore not be made. However, the results of the current study, together with the spectrum of activity of RU 64004 against aerobes, suggest clinical potential in the treatment of non-life-threatening mixed anaerobic infections (such as those encountered in the ear, nose, and throat; in skin and soft tissue, including bite wounds; and in bacterial vaginosis) instead of orally administered agents such as clindamycin, amoxicillin-clavulanate, or cephalosporins. Conclusions as to the biological potency of RU 64004 will also depend on toxicology, pharmacokinetics, and animal studies.

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