ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1986, p. 789–791
0066-4804/86/110789-03$02.00/0

Vol. 30, No. 5

Comparative Activity of β-Lactamase Inhibitors YTR 830, Clavulanate, and Sulbactam Combined with β-Lactams against β-Lactamase-Producing Anaerobes

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Received 2 June 1986/Accepted 26 August 1986

The in vitro activities of the β-lactamase inhibitors YTR 830, clavulanate, and sulbactam combined with six β-lactams against 88 β-lactamase-producing anaerobes were determined. When combined with the β-lactams, the three β-lactamase inhibitors showed no synergy against the 10 *Bacteroides fragilis* homology group II strains. When the β-lactams were combined with the inhibitors, their geometric mean MICs against the remaining 78 strains were reduced from 4.2 to 150.2 μg/ml to 0.2 to 12.9 μg/ml. The activity of the β-lactams combined with the β-lactamase inhibitors was significantly greater than that of the β-lactams alone against all groups except *B. fragilis* homology group II, with 76 to 100% of the strains susceptible to ampicillin plus inhibitor and ≥90% susceptible to the other combinations.

Although the antimicrobial susceptibility spectrum of anaerobes has remained relatively stable, β-lactamase-mediated resistance to penicillin and other β-lactams has been described for species in the *Bacteroides fragilis* group and for other *Bacteroides* spp., *Fusobacterium nucleatum*, and *Clostridium butyricum* (3, 7, 15, 18, 19). Several studies have demonstrated that the β-lactamase inhibitors clavulanate and sulbactam act synergistically in vitro with β-lactams against β-lactamase-positive, but not β-lactamase-negative, anaerobic organisms (2, 4, 5, 16, 20, 21). YTR 830 is a new β-lactamase inhibitor (1) with activity comparable to that of clavulanate and superior to that of sulbactam against β-lactamase-producing members of the family *Enterobacteriaceae* (6, 10). The purpose of this study was to compare the in vitro activities of six β-lactams (ampicillin, ticarcillin, mezlocillin, piperacillin, apalcillin, and cefoperazone) alone and in combination with fixed concentrations of three β-lactamase inhibitors (YTR 830, clavulanate, and sulbactam) against a spectrum of β-lactamase-producing anaerobes.

(This research was presented in part at the 86th Annual Meeting of the American Society for Microbiology [P. C. Appelbaum, S. K. Spangler, M. R. Jacobs, M. E. Walker, and S. Yamabe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, A88, p, 15].)

Eighty-eight β-lactamase-producing anaerobic strains were tested. These included 21 *B. fragilis* homology group I strains, 10 *B. fragilis* homology group II strains, 29 *B. fragilis* group strains (15 *B. thetaiotaomicron* strains, 5 *B. ovatus* strains, 5 *B. distasonis* strains, 2 *B. vulgatus* strains, 1 *B. uniformis* strain, and 1 *Bacteroides* strain 3452A), 15 *Bacteroides* spp. strains (1 *B. melaninogenicus* strain, 1 *B. asaccharolyticus* strain, 2 *B. oralis* strains, 2 *B. bivius* strains, 2 *B. disiens* strains, and 7 *B. oris*/*B. buccae* strains), and 13 fusobacteria (3 *F. nucleatum* strains, 6 *F. varium* strains, and 4 *F. mortiferum* strains). All were identified by conventional means with prereduced anaerobically sterilized media and gas-liquid chromatography (8). The homology status of the *B. fragilis* strains had been determined by J. Johnson, Virginia Polytechnic Institute and State University, Blacksburg, by DNA immobilization on nitrocellulose filters and DNA competition experiments, using *B. fragilis* ATCC 25285 as the neotype strain of homology group I (11). The presence of β-lactamase was detected by a chromogenic cephalosporin method on growth from enriched blood agar plates by using nitrocefin disks (BBL Microbiology Systems, Cockeysville, Md.), in accordance with standard practice (4, 10). Organisms were stored at −70°C in thioglycolate-glycerol (85:15, vol/vol) and were subcultured on enriched blood agar plates before testing.

Laboratory reference powders of the following agents were used: ticarcillin and clavulanate (Beecham Laboratories, Bristol, Tenn.); mezlocillin (Miles Laboratories, Inc., West Haven, Conn.); piperacillin (Lederle Laboratories, Pearl River, N.Y.); apalcillin (Wyeth Laboratories, Philadelphia, Pa.); cefoperazone and sulbactam (Pfizer Inc., New York, N.Y.); ampicillin (Bristol-Myers Laboratories, Wallingford, Conn.); cefoxitin (Merck Sharpe & Dohme, West Point, Pa.); and YTR 830 (Taiho Pharmaceuticals, Tokyo, Japan). The powders were dissolved and diluted in accordance with the instructions of the manufacturers, and the potency of the agents was checked by determination of the MICs for quality control strains.

Susceptibility testing of the isolates was performed by agar dilution on Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.) (14, 17). Plates contained serial doubling dilutions of the β-lactams (ampicillin, 0.125 to 256 μg/ml; ticarcillin, mezlocillin, piperacillin, apalcillin, and cefoperazone, 0.125 to 512 μg/ml) alone and with a fixed concentration (2 μg/ml) of each β-lactamase inhibitor. Plates containing serial doubling dilutions of each inhibitor alone (1 to 32 μg/ml), cefoxitin (1 to 64 μg/ml), and aerobic and anaerobic growth controls were also included. For strains other than those in the *B. fragilis* group, the Wilkins-Chalgren medium was supplemented with 5% sterile defibrinated sheep blood. Plates were prepared within 1 week of use and prereduced in an anaerobic glove box (Coy Laboratory Products, Ann

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TABLE 1. Activity of β-lactams alone and combined with inhibitors

Drug or inhibitor	Activity of:									
	B. fragilis homology group I (21)[a]		*B. fragilis* homology group II (10)		*B. fragilis* group (29)		*Bacteroides* spp. other than *B. fragilis* (15)		*Fusobacterium* spp. (13)	
	%[b]	MIC[c]	%	MIC	%	MIC	%	MIC	%	MIC
Ampicillin (4[d])	14	68.4	30	11.3	24	32.8	40	4.2	23	27.3
+YTR 830	86	1.0	40	7.0	100	0.7	100	0.2	85	1.4
+Sulbactam	76	1.3	40	8.0	93	0.7	100	0.2	92	2.4
+Clavulanate	90	0.4	60	5.7	100	0.5	100	0.2	92	1.4
Ticarcillin (64)	57	73.0	100	16.0	72	27.7	100	10.6	77	16.0
+YTR 830	100	1.7	100	7.0	100	1.5	100	1.4	100	1.1
+Sulbactam	100	1.4	100	6.5	100	1.7	100	0.7	92	2.9
+Clavulanate	100	1.2	100	8.0	100	1.0	100	0.7	100	1.2
Mezlocillin (64)	57	92.0	100	9.2	68	29.7	100	4.8	38	150.2
+YTR 830	100	2.4	100	4.3	100	4.6	100	0.7	100	6.5
+Sulbactam	100	3.9	100	4.3	100	6.4	100	0.9	100	12.9
+Clavulanate	100	2.9	100	4.6	100	5.8	100	0.9	92	7.2
Piperacillin (64)	62	35.3	100	4.9	86	20.3	100	6.7	62	67.5
+YTR 830	100	1.5	100	2.3	100	3.4	100	0.7	100	3.8
+Sulbactam	100	2.1	100	2.1	97	5.1	100	0.8	92	8.0
+Clavulanate	100	1.4	100	2.6	100	4.8	100	0.7	92	3.4
Apalcillin (64)	57	56.1	100	18.4	62	51.6	100	11.0	62	57.5
+YTR 830	100	9.4	100	8.6	100	5.7	100	0.8	92	4.7
+Sulbactam	100	4.7	100	10.6	100	6.0	100	0.9	92	8.4
+Clavulanate	100	4.3	100	9.9	100	5.7	100	0.9	92	4.2
Cefoperazone (32)	10	141.3	90	19.7	52	45.8	93	6.4	77	18.8
+YTR 830	95	6.8	100	12.1	100	6.0	100	1.4	100	3.1
+Sulbactam	90	6.8	90	16.0	100	6.5	100	1.4	100	5.0
+Clavulanate	100	5.0	90	16.0	100	5.7	100	1.6	100	5.0
Cefoxitin (16)	100	6.4	60	24.3	97	6.5	100	2.4	100	1.9
YTR 830	—[e]	16.6	—	13.0	—	24.6	—	22.1	—	>32
Sulbactam	—	15.5	—	17.1	—	22.4	—	25.4	—	>32
Clavulanate	—	15.5	—	29.9	—	30.5	—	22.1	—	>32

[a] Number of strains tested.
[b] Percentage of strains susceptible to MIC breakpoint.
[c] Geometric mean MIC (μg/ml).
[d] MIC breakpoint (μg/ml).
[e] —, Not applicable.

Arbor, Mich.) immediately before inoculation. The activity of the β-lactams and β-lactamase inhibitors in media was checked for each run by testing with *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741 (β-lactamase positive), and *Clostridium perfringens* ATCC 13124 (β-lactamase negative). Each set of organisms was inoculated onto a complete set of all antimicrobial combinations from the same set of inocula to ensure comparable β-lactamase production. MICs were interpreted in accordance with published breakpoints (13, 17). Synergy was defined as a fourfold or greater decrease in the MIC of the β-lactam in the combination compared with that of the β-lactam alone.

The MICs of the β-lactamase inhibitors alone were all ≥8 μg/ml, with most being ≥32 μg/ml; therefore, the inhibitors were present at less than or equal to one-fourth of the MIC. When combined with the β-lactams, the three β-lactamase inhibitors showed no synergy against the 10 *B. fragilis* homology group II strains. When the β-lactams were combined with the inhibitors, their MICs for 90% of strains tested against the remaining 78 strains were reduced from ≥16 μg/ml to ≤16 μg/ml. The percentages of the strains susceptible to the breakpoint drug concentrations and the geometric mean MICs are shown in Table 1. Against the penicillins alone, 14 to 40% of the anaerobe groups were susceptible to ampicillin, 57 to 100% were susceptible to ticarcillin and apalcillin, 38 to 100% were susceptible to mezlocillin, and 62 to 100% were susceptible to piperacillin. Cefoperazone was active against only 10% of the *B. fragilis* homology group I strains and was active against 52 to 93% of the strains in the other groups. Cefoxitin was active against 97 to 100% of the strains except those in *B. fragilis* homology group II (60%). The activity of the β-lactams combined with the β-lactamase inhibitors was significantly greater than that of the β-lactams alone against all groups except *B. fragilis* homology group II, with 76 to 100% of the strains susceptible to the ampicillin-inhibitor combinations and ≥90% susceptible to the other combinations. No differences among the three β-lactamase inhibitors were found. The β-lactamase

inhibitors did not affect the activity of the β-lactams against *B. fragilis* homology group II, although this group was susceptible to the broad-spectrum penicillins alone.

This study confirms previous findings of synergy between clavulanate and sulbactam combined with β-lactams against β-lactamase-producing anaerobes (2, 4, 5, 16, 20, 21). In the current study, YTR 830 exhibited similar activity to that of sulbactam and clavulanate and no significant differences among the three inhibitors were noted. *B. fragilis* group II strains (9, 11, 12) have been shown to be more susceptible to penicillin G and cephalothin than group I strains are, but less susceptible to cefoxitin; it has also been found that penicillin or cephalothin combined with clavulanate does not show synergy against group II strains (21). The present study confirmed these findings and showed that YTR 830 and sulbactam similarly have no synergistic activity.

Overall, β-lactams combined with β-lactamase inhibitors show excellent activity against most β-lactamase-producing anaerobes. If the results of toxicity and pharmacokinetic studies are favorable, YTR 830 may prove useful in combination with a β-lactam in the therapy of mixed aerobic-anaerobic infections; clavulanate and sulbactam may also be used for this purpose. A clinical role for these combinations awaits clinical studies.

This study was supported in part by a grant from Taiho Pharmaceuticals, Tokyo, Japan.

The following individuals are thanked for kind provision of cultures: Cindy Kaufmann, Johns Hopkins University Hospitals, Baltimore, Md.; Itzhak Brook, Armed Forces Radiobiology Institute, Bethesda, Md.; George Lombard, Centers for Disease Control, Atlanta, Ga.; Francis Tally, New England Medical Center, Boston, Mass.; Tracy Wilkins, Lillian Holdeman Moore, and John Johnson, Virginia Polytechnic Institute and State University, Blacksburg; Sydney Finegold, Wadsworth Veterans Administration Hospital, Los Angeles, Calif.; Jon Rosenblatt, Mayo Clinic, Rochester, Minn.; and Madeleine Sebald, Pasteur Institute, Paris, France.

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