ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1983, p. 429–431
0066-4804/83/090429-03$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 24, No. 3

In Vitro Activity of Amoxicillin in Combination with Clavulanic Acid Against *Mycobacterium tuberculosis*

MICHAEL H. CYNAMON* AND GREGORY S. PALMER

Department of Medicine, Veterans Administration Medical Center, and Department of Medicine, State University of New York Upstate Medical Center, Syracuse, New York 13210

Received 21 March 1983/Accepted 17 June 1983

The comparative in vitro activity of amoxicillin alone and in combination with clavulanic acid against 15 isolates of *Mycobacterium tuberculosis* was evaluated by broth dilution susceptibility testing. Amoxicillin inhibited 4 of 15 isolates at 8 μg/ml or less but was not bactericidal against any of the isolates at that concentration. Amoxicillin in combination with clavulanic acid was bactericidal for 14 of 15 isolates tested at an amoxicillin concentration of 4 μg/ml or less and a clavulanic acid concentration of 2 μg/ml or less.

Mycobacterium tuberculosis produces a constitutive, intracellular β-lactamase which has activity as both a penicillinase and a cephalosporinase (3). This enzyme appears to have a role in determining the resistance of *M. tuberculosis* to β-lactamase-labile β-lactams (2). There has been relatively little study of the in vitro activity of β-lactams against mycobacteria in general and against *M. tuberculosis* in particular. The purpose of this study was to compare the in vitro activity of amoxicillin alone and in combination with clavulanic acid, a β-lactamase inhibitor, against *M. tuberculosis*.

The isolates were from clinical specimens submitted to the microbiology laboratory at the Veterans Administration Medical Center, Syracuse, N.Y., and confirmed as *M. tuberculosis* by the Reference Laboratory for Tuberculosis and Other Mycobacterial Diseases, Veterans Administration Medical Center, West Haven, Conn. (strains BIE, BON, BRE, DGN, DLV, DUH, JNS, and MEE) or from Howard Gruft, Division of Laboratories and Research, New York State Department of Health, Albany (strains 1268, 1540, 1560, 1578, 1694, 1789, and 1790).

The strains of *M. tuberculosis* were tested for β-lactamase activity with nitrocefin (provided by Glaxo Research, Greenford, Middlesex, United Kingdom) as described in a previous paper (1) except that the culture medium was 7H10 broth. All of the strains of *M. tuberculosis* used in this study had β-lactamase activity.

The antimicrobial agents evaluated in this study were provided as standard powders as follows: amoxicillin (potency 860 μg/mg) and potassium clavulanate (potency, 826 μg/mg) (Beecham Laboratories, Bristol, Tenn.). Stock solutions of each antimicrobial agent were prepared immediately before use by hydrating a known weight of drug in Middlebrook and Cohn 7H10 broth (5) with Middlebrook OADC Enrichment (Difco Laboratories, Detroit, Mich.) and 0.05% Tween 80, followed by filter sterilization through a GA-6 0.45-μm membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.).

The mycobacteria were grown in 7H10 broth at 37°C on a rotary shaker and subcultured 3 days before use. The cultures were diluted in 7H10 broth to yield 5 Klett units per ml (Klett-Summerson colorimeter, Klett Manufacturing, Brooklyn, NY). These were used to inoculate 7H10 broth containing the following concentration (micrograms per milliliter) of drug(s): amoxicillin, 8; amoxicillin, 4; amoxicillin 2; clavulanic acid, 8; clavulanic acid, 4; amoxicillin, 8, and clavulanic acid, 4; amoxicillin, 4, and clavulanic acid, 2; amoxicillin, 2, and clavulanic acid, 1; amoxicillin, 1, and clavulanic acid, 0.5; and amoxicillin, 0.5, and clavulanic acid, 0.25. A control tube without any drug was run for each mycobacterial strain. The titers of the mycobacteria were determined in duplicate on 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.). The final concentration of organisms was 0.1 Klett unit per ml, or about 5×10^4 CFU/ml (range, 1×10^4 to 7.7×10^4 CFU/ml).

The optical density at 550 nm (OD_{550}) of the above suspensions was determined with a Spectrophotometer Modernization System model 252 (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) of a DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The OD_{550} of the inoculated tubes was not measurably different from that of the medium blank. The cultures were incubated for 7 days at 37°C on a rotary shaker. If the OD_{550} of the control tubes (those without antibiotic) was ≥0.050, the antibiotic-containing tubes were read. The antibiotic-containing tubes were considered to be inhib-

TABLE 1. OD_{550} for antibiotic and control cultures

Strain	Control	OD_{550} for following antibiotic[a] at indicated concn (μg/ml):									
		A (8)	A (4)	A (2)	C (8)	C (4)	A (8)-C (4)	A (4)-C (2)	A (2)-C (1)	A (1)-C (0.5)	A (0.5)-C (0.25)
1268	0.583	0.000	0.000	0.022	0.117	0.419	0.000	0.000	0.000	0.000	0.020
1540	0.798	0.003	0.200	0.412	0.526	0.771	0.000	0.000	0.000	0.002	0.277
1560	0.431	0.287	0.352	0.435	0.497	0.452	0.000	0.000	0.002	0.054	0.447
1578	0.570	0.350	0.421	0.374	0.521	0.556	0.000	0.000	0.000	0.129	0.232
1694	0.467	0.000	0.080	0.222	0.349	0.516	0.000	0.000	0.000	0.000	0.057
1789	0.740	0.290	0.609	0.440	0.565	0.740	0.000	0.000	0.000	0.020	0.340
1790	0.727	0.158	0.380	0.420	0.612	0.720	0.000	0.000	0.000	0.020	0.231
BIE	0.514	0.280	0.418	0.539	0.446	0.548	0.000	0.000	0.000	0.040	0.289
BON	0.909	0.225	0.440	0.383	0.650	0.841	0.000	0.000	0.002	0.096	0.114
BRE	0.576	0.409	0.638	0.505	0.595	0.677	0.000	0.000	0.003	0.114	0.440
DGN	0.974	0.681	0.702	0.894	0.682	0.692	0.000	0.000	0.004	0.625	0.747
DLV	0.660	0.000	0.040	0.033	0.079	0.355	0.000	0.000	0.000	0.025	0.026
DUH	0.150	0.031	0.048	0.128	0.043	0.060	0.000	0.000	0.000	0.022	0.024
JNS	0.512	0.080	0.105	0.106	0.140	0.323	0.000	0.000	0.006	0.046	0.248
MEE	0.628	0.020	0.048	0.048	0.127	0.415	0.000	0.000	0.000	0.010	0.015

[a] A, Amoxicillin; C, clavulanic acid.

ited if their OD_{550} was ≤0.010. A 100-μl sample from the inhibited tubes was plated in duplicate on 7H10 agar plates to determine the bactericidal activity. The plates were incubated for 4 weeks at 37°C. An antibiotic was considered to be bactericidal if there was a 99% reduction in the number of CFU per milliliter.

The OD_{550} of the control cultures ranged from 0.150 to 0.974 (Table 1). Clavulanic acid did not inhibit any of the strains tested at 8 μg/ml, the highest concentration used. Amoxicillin inhibited 4 of 15 strains tested at 8 μg/ml (Table 1). Amoxicillin alone was not bactericidal for any of the strains at 8 μg/ml. The combination of amoxicillin-clavulanic acid inhibited all of the strains tested at a concentration of 2 μg of amoxicillin per ml and 1 μg of clavulanic acid per ml. Amoxicillin-clavulanic acid was bactericidal for 14 of 15 strains of *M. tuberculosis* at a concentration of 4 μg of amoxicillin per ml and 2 μg of clavulanic acid per ml (Tables 2 and 3). Strain 1578 was just shy of 99% killing at the highest concentration of amoxicillin-clavulanic acid used. When the OD_{550} of a culture was greater than 0.000, in no instance was there bactericidal activity; however, not surprisingly, there were cultures in which the OD_{550} was 0.000 and killing was not achieved.

The β-lactamase of *M. tuberculosis* appears to play a role in determining the in vitro response to β-lactamase-labile β-lactams. Kasik (2) demonstrated that concentrations exceeding 100 μg of the β-lactamase-susceptible penicillins (penicillin G, penicillin V, or ampicillin) per ml were needed to completely inhibit growth of the R1 RV strain of *M. tuberculosis*. The β-lactamase-resistant compounds (nafcillin, oxacillin, and cephalothin) inhibited growth at a concentration of 25 μg/ml. In addition, oxacillin was found to inhibit mycobacterial β-lactamase activity in cultures and in a crude cell-free system. In a subsequent study, Kasik et al. (4) demonstrated that only 1 of 32 isolates of *M. tuberculosis* was inhibited at 10 μg of benzylpenicillin per ml. Of the remaining isolates, 6 were inhibited by 100 μg/ml, and the other 25 were not inhibited at this concentration. The combination of amoxicillin-clavulanic acid achieved 99% killing with 14 of

TABLE 2. Bactericidal activity of amoxicillin-clavulanic acid

Strain	Initial titer ($\times 10^4$)	CFU/plate for bactericidal activity	CFU/plate with following antibiotic[a] combination:		
			A (8)[b]-C (4)	A (4)[b]-C (2)	A (2)[b]-C (1)
1268	2.0	20	0, 0	0, 0	2, 3
1540	1.0	10	4, 0	2, 1	15, 9
1560	2.9	29	1, 0	1, 2	72, 123
1578	1.5	15	19, 15	40, 86	>100
1694	1.4	14	3, 3	9, 2	12, 23
1789	1.0	10	1, 2	4, 3	>100
1790	7.7	77	1, 2	3, 0	64, 53
BIE	5.4	54	11, 8	10, 7	>100
BON	1.2	12	6, 4	11, 9	>200
BRE	5.7	57	5, 0	5	>100
DGN	4.9	49	0, 1	2, 3	>200
DLV	7.2	72	9, 5	4, 3	12, 6
DUH	7.4	74	6, 7	38, 20	>200
JNS	2.2	22	5, 13	18, 28	>200
MEE	5.4	54	6, 6	21, 35	>100

[a] A, Amoxicillin; C, clavulanic acid.

[b] Numbers in parentheses represent antibiotic concentration in micrograms per milliliter. Pairs of numbers (<100) represent duplicate plating, except for strain BRE (amoxicillin, 4 μg/ml; clavulanic acid, 2 μg/ml), for which there was contamination of the second plate.

TABLE 3. Activity of amoxicillin (A) or amoxicillin-clavulanic acid (A-C) against *M. tuberculosis*

Strain	MIC[a] (μg/ml)		MBC[b] (μg/ml)	
	A	A-C	A	A-C
1268	4	1-0.5	>8	1-0.5
1540	8	1-0.5	>8	4-2
1560	>8	2-1	>8	4-2
1578	>8	2-1	>8	>8-4
1694	8	1-0.5	>8	4-2
1789	>8	2-1	>8	4-2
1790	>8	2-1	>8	2-1
BIE	>8	2-1	>8	4-2
BON	>8	2-1	>8	4-2
BRE	>8	2-1	>8	4-2
DGN	>8	2-1	>8	4-2
DLV	8	2-1	>8	2-1
DUH	>8	2-1	>8	4-2
JNS	>8	2-1	>8	4-2
MEE	>8	2-1	>8	4-2

[a] MIC, Minimal inhibitory concentration.
[b] MBC, Minimal bactericidal concentration.

15 strains at a concentration of 4 μg of amoxicillin per ml and 2 μg of clavulanic acid per ml. Amoxicillin alone did not achieve killing at 8 μg/ml, the highest concentration tested. The addition of clavulanic acid to amoxicillin improves its inhibitory and bactericidal activity. The mechanism for the improved activity of amoxicillin is likely due to the inhibition of mycobacterial β-lactamase activity by clavulanic acid.

Further in vitro study of the activity of amoxicillin-clavulanic acid against *M. tuberculosis* seems to be warranted by the results of our study. It is not clear how effective the combination would be in animal models of tuberculosis or in patients. This antibacterial combination may be useful in the treatment of clinical tuberculosis. The potential efficacy of this combination and its availability as an oral agent make further in vitro and in vivo evaluation attractive.

LITERATURE CITED

1. **Cynamon, M. H., and G. S. Palmer.** 1982. In vitro susceptibility of *Mycobacterium fortuitum* to *N*-formimidoyl thienamycin and several cephamycins. Antimicrob. Agents Chemother. **22**:1079–1081.
2. **Kasik, J. E.** 1965. Activity of some semisynthetic penicillins on *Mycobacterium tuberculosis*, p. 315–302. Antimicrob. Agents Chemother. 1964.
3. **Kasik, J. E.** 1979. Mycobacterial β-lactamases, p. 339–350. *In* J. M. T. Hamilton-Miller and J. T. Smith (ed.), β-Lactamases. Academic Press, Inc., New York.
4. **Kasik, J. E., M. Monick, and B. Schwarz.** 1980. β-Lactamase activity in slow-growing nonpigmented mycobacteria and their sensitivity to certain β-lactam antibiotics. Tubercle **61**:213–219.
5. **Vestal, A. L.** 1969. Procedures for the isolation and identification of mycobacteria. Public Health Service publication no. 1995. Laboratory Division, National Communicable Disease Center, Atlanta, Ga.