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Inhibition of β-Lactamase in *Neisseria gonorrhoeae* by Sodium Clavulanate

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Sodium clavulanate at subinhibitory concentrations affected the activity of penicillin G, ampicillin, or amoxicillin on β-lactamase-positive strains of *Neisseria gonorrhoeae* as demonstrated by marked reduction in the minimal inhibitory concentrations of the drugs for the organisms. The compound did not affect the activity of these penicillins on β-lactamase-negative strains of *N. gonorrhoeae*. It also had no effect on the activity of cefoxitin against either β-lactamase-negative or -positive strains. The reduction in minimal inhibitory concentrations of the penicillins for the β-lactamase-positive organisms brought about by sodium clavulanate is probably due to inhibition of the β-lactamase by the compound.

β-Lactamase-producing (β-lac$^+$) *Neisseria gonorrhoeae* were recognized in 1976 (1, 4, 10, 11). Because β-lactamase almost always makes strains resistant to penicillin, it has been necessary to alter the therapeutic regimen for "uncomplicated gonorrhea" caused by these strains from the presently recommended 4.8×10^6 U of aqueous procaine penicillin and 1 g of probenicid (3) to 2 g of spectinomycin, intramuscularly (4).

Sodium clavulante (Fig. 1) is a microbial metabolite of *Streptomyces clavuligarus* which irreversibly inhibits certain β-lactamases in vitro, and because it inhibits these enzymes, it potentiates the effect of penicillins against β-lactamase-producing organisms such as staphylococci and *Enterobacteriaceae* (12). Since penicillin is the preferred drug for treatment of gonorrhea caused by β-lactamase-negative (β-lac$^-$) *N. gonorrhoeae*, we were interested in determining whether the sodium clavulanate potentiation of penicillin activity occurred with β-lac$^+$ *N. gonorrhoeae* and whether the resulting activity was similar to that in β-lac$^-$ strains. Therefore, we studied the effects of sodium clavulanate on the MICs of penicillin, ampicillin, amoxicillin, which are susceptible to gonococcal β-lactamase, and another β-lactam, cefoxitin, which is not susceptible to this enzyme.

A total of 83 strains of *N. gonorrhoeae*, isolated in various countries, were randomly selected for the study. They were tested for β-lactamase production by the chromagenic cephalosporin procedure (9, 13), and 36 strains were positive for β-lactamase.

Sodium clavulanate, penicillin G, ampicillin, amoxicillin, and cefoxitin were reconstituted according to manufacturer's specifications, and, with the exception of sodium clavulanate, further dilutions were prepared in water. Sodium clavulanate dilutions were prepared in phosphate buffer (pH 7.0). The media, reagents, and procedure for the agar dilution method of antibiotic susceptibility testing of *N. gonorrhoeae* have been described (2). Minimal inhibitory concentrations (MICs) were determined for each antibiotic alone and for penicillin G, ampicillin, amoxicillin, and cefoxitin in the presence of sodium clavulanate.

In a preliminary study of a smaller group of strains, it was determined that the MICs for sodium clavulanate ranged from 1 to 5 μg/ml. Therefore, a subinhibitory concentration of 0.5 μg/ml was used in the tests with the other antibiotics. In later studies, occasional strains were inhibited by 0.5 μg/ml, but these strains were eliminated from the study.

The ranges and median MICs for both β-lactamase-negative and -positive strains of penicillin, ampicillin, amoxicillin, and cefoxitin, alone and in the presence of sodium clavulanate, as well as MICs of sodium clavulanate, are shown in Table 1. The median MICs of penicillin, ampicillin, and amoxicillin for the β-lactamase-positive strains were all >8 μg/ml, but in the presence of sodium clavulanate, the MICs were reduced more than 32-fold to 0.25 μg/ml. However, sodium clavulanate did not have an effect on the MICs of the drugs for the β-lactamase-negative strains. The median MICs were low and did not change in the presence of sodium clavulanate. The effect of sodium clavulanate on the antibacterial activity of cefoxitin was minimal with either the β-lactamase-positive or -negative strains.

Similar results showing the effect of sodium clavulanate on penicillin, ampicillin, amoxicillin,

carbenicillin, cephaloridine, and cephalothin have been reported (12, 14). The organisms used in these studies were staphylococci, *Enterobacteriaceae,* and *Pseudomonas.* The failure of sodium clavulanate to affect the activity of cefoxitin on *Bacteroides* has also been reported (14).

The β-lactamase produced by gonococci is the TEM type (6), and it is active on the penicillins used in this study, but it is not active on cefoxitin (C. Thornsberry, J. W. Biddle, P. L. Perine, and M. S. Siegel, Proceedings of the Conference on the Immunobiology of *Neisseria gonorrhoeae,* in press). Since the sodium clavulanate reduced the MICs of the penicillins for the β-lactamase-producing strains but had no effect on the activity of the β-lactamase-negative strains, and since it had no effect on the MICs of cefoxitin, it seems probable that the compound inhibited the β-lactamase of the organisms that were capable of producing it.

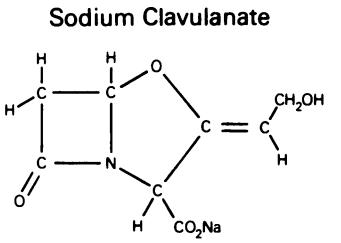

FIG. 1. *Structure of sodium clavulanate.*

Studies on the activity of various concentrations of sodium clavulanate and penicillin on β-lactamase-negative and -positive strains were also performed. The concentrations of each drug used in these tests are shown in Tables 1 and 2. Unique patterns were obtained for the β-lactamase-positive strains as compared to the β-lactamase-negative strains. An example of the results obtained with one of each of these strains is shown in Tables 2 and 3. With the β-lactamase-negative strain (Table 2), the organism is inhibited by 0.12 or 0.25 μg of penicillin per ml

TABLE 1. *Effect of sodium clavulanate on the MICs of four β-lactam antibiotics for β-lactamase-negative and positive strains of N. gonorrhoeae*

Drug	MIC (μg/ml)			
	β-Lactamase positive		β-Lactamase negative	
	Range	Median	Range	Median
CA[a]	1–5		1–5	
Penicillin	2.0–>8	>8	0.015–4.0	0.5
Penicillin + CA[a]	0.015–1.0	0.25	0.015–4.0	0.5
Ampicillin	4.0–>8	>8	0.015–2.0	0.25
Ampicillin + CA[a]	0.015–1.0	0.25	0.0075–2.0	0.25
Amoxicillin	4.0–>8	>8.0	0.03–2.0	0.25
Amoxicillin + CA[a]	0.03–1.0	0.25	0.03–1.0	0.25
Cefoxitin	0.06–>8	1.0	0.12–>8	1.0
Cefoxitin + CA[a]	0.06–2	0.5	0.06–>8	0.5

[a] CA, Sodium clavulanate, was used at 0.5 μg/ml, and the antibiotic concentrations were varied in $\log_2$ dilution steps. Occasional strains had sodium clavulanate MICs of 0.5 μg/ml and were not used in the study.

TABLE 2. *Action of various concentrations of sodium clavulanate and penicillin on a β-lactamase-negative strain of N. gonorrhoeae*

Clavulanate concn (μg/ml)	Penicillin concn (μg/ml)[a]													
	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.015	0.0075	0.0035	0
4	–	–	–	–	–	–	–	–	+	+	+	+	+	+
2	–	–	–	–	–	–	–	+	+	+	+	+	+	+
1	–	–	–	–	–	–	–	+	+	+	+	+	+	+
0.5	–	–	–	–	–	–	–	+	+	+	+	+	+	+
0.25	–	–	–	–	–	–	–	+	+	+	+	+	+	+
0.12	–	–	–	–	–	–	–	+	+	+	+	+	+	+
0.06	–	–	–	–	–	–	–	+	+	+	+	+	+	
0	–	–	–	–	–	–	–	+	+	+	+	+	+	

[a] –, No growth; +, growth.

TABLE 3. *Action of various concentrations of sodium clavulanate and penicillin on a β-lactamase-positive strain of Neisseria gonorrhoeae*

Clavulanate concn (μg/ml)	Penicillin concn (μg/ml)[a]													
	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.015	0.0075	0.0035	0
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1	−	−	−	−	−	−	−	−	−	−	+	+	+	+
0.5	−	−	−	−	−	−	−	−	−	+	+	+	+	+
0.25	−	−	−	−	−	−	−	−	+	+	+	+	+	+
0.12	−	−	−	−	−	−	−	+	+	+	+	+	+	
0.06	−	−	−	−	−	−	+	+	+	+	+	+	+	+
0	−	−	+	+	+	+	+	+	+	+	+	+	+	+

[a] −, No growth; +, growth.

at all concentrations of sodium clavulanate, indicating that the latter compound had little or no effect on action of penicillin. However, with the β-lactamase-positive strain (Table 3), increasing the amount of sodium clavulanate increased the activity of penicillin. Of the strains tested in this manner, all the β-lactamase-negative strains showed patterns similar to one shown in Table 2, and β-lactamase-positive strains showed patterns similar to those shown in Table 3.

Gonorrhea is epidemic in the world today, and the approximately one million cases reported in the United States for 1976 (5) probably represent only a portion of the actual incidence. Although during the 20 years preceding 1972 the organisms had generally become relatively more resistant to penicillin, this trend was then arrested (7), and gonococci were still considered to be clinically susceptible to penicillin. However, in 1976 some gonococci were shown to produce β-lactamase (1, 4, 10, 11) mediated by a plasmid (6).

Because of the epidemicity of gonorrhea, and because sodium clavulanate appears to inhibit the gonococcal β-lactamase, it might prove to be a useful drug in combination with penicillin for treatment of gonorrhea caused by β-lac$^+$ gonococci. This would be desirable because penicillin has been shown to cure more than 97% of β-lac$^-$ gonorrhea (8) but generally fails to cure β-lac$^+$ gonorrhea.

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