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In Vitro Susceptibility of *Neisseria gonorrhoeae* to Spectinomycin Examined by a Broth Dilution Method

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The in vitro susceptibility of 113 strains of *Neisseria gonorrhoeae* to spectinomycin was determined by the broth dilution method and was compared with their susceptibility to penicillin and ampicillin. All strains demonstrated a susceptibility to spectinomycin in a range between 2.5 and 20 μg/ml; 96% of these strains were susceptible in a range from 5 to 10 μg/ml. The same strains were susceptible to ampicillin at concentrations between 0.06 and 0.5 μg/ml and to penicillin between 0.02 and 1.25 units/ml. For the majority of strains, the minimal inhibitory and bactercidal concentrations were identical; however, with more than 10% of the strains, the minimal inhibitory concentration was lower than the minimal bactericidal concentration.

Spectinomycin, under the name of actinospectacin, was introduced 10 years ago in the United Kingdom for treatment of gonorrhea (2, 6). In this country, spectinomycin has also been used in clinical trials (4, 5). Its lack of cross-resistance and cross-allergenicity with penicillin made this antibiotic a very promising choice for the single-dose treatment of gonorrhea. Spectinomycin was approved in 1971 by the Food and Drug Administration for treatment of gonorrhea, and wide usage of this drug may be expected. A comprehensive clinical evaluation of spectinomycin has recently been published (3).

The minimal inhibitory concentration (MIC) of antibiotics for treatment of *Neisseria gonorrhoeae* infection is, in the majority of cases, determined by the agar dilution technique. We have used the broth dilution technique to determine the in vitro susceptibility of *N. gonorrhoeae* to spectinomycin. This method has the advantage of allowing, in addition to the determination of the MIC, the examination of the minimal bactericidal activity. Susceptibility to penicillin and ampicillin, the drugs until now most commonly used in treatment of gonorrhea, was used for comparison. (A report of this work was presented at the 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., April 1972.)

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MATERIALS AND METHODS

The 113 strains of *N. gonorrhoeae* used were isolated from clinical specimens obtained from male and female patients seen in the clinics of the E. J. Meyer Memorial Hospital, Buffalo, N.Y. The identification of *N. genorrhoeae* was based on the morphology of the colony on chocolate agar at 37 C, appearance on a gram-stained film, lack of growth on Endo agar or on nutrient agar at 37 C, lack of growth on chocolate agar at 22 C, positive oxidase reaction, and glucose fermentation. Cultures grown for 24 h on chocolate agar were used for the incoulation of 5 ml of Mueller-Hinton broth kept in screw-capped tubes containing glass beads. A bacterial suspension was prepared by shaking the culture on a Vortex shaker and incubating it for 7 to 8 h in a CO_2 incubator. The turbidity of the suspension was adjusted to either 9×10^8 or 12×10^9 organisms by use of McFarland standards. Drug dilutions were made in Mueller-Hinton broth in 0.5-ml portions to which 0.05 ml of the bacterial suspension was added. The tubes were kept in a CO_2 incubator for 24 h, and then the results were read. The last drug dilution in which no growth was visible was considered the MIC. The tube contents were then subcultured by placing a drop on a chocolate agar plate. The last drug dilution from which no viable organisms were recovered was recorded as the minimal bactericidal concentration (MBC).

RESULTS

The MIC and MBC of the antibiotics used are summarized in Table 1. All of the strains examined demonstrated susceptibility to spectin-

TABLE 1. *Minimal inhibitory and bactericidal concentrations (MIC and MBC) of antibiotics for 113 strains of N. gonorrhoeae*

Antibiotic (concn)	No. of strains	
	MIC	MBC
Spectinomycin		
20 μg/ml	1	4
10 μg/ml	73	85
5 μg/ml	36	23
2.5 μg/ml	3	1
Ampicillin		
0.5 μg/ml	2	4
0.25 μg/ml	45	60
0.12 μg/ml	61	44
0.06 μg/ml	5	5
Penicillin		
1.25 units/ml	0	4
0.63 units/ml	18	33
0.31 units/ml	51	39
0.16 units/ml	20	18
0.08 units/ml	16	13
0.04 units/ml	3	3
0.02 units/ml	5	3

omycin in a range between 2.5 and 20 μg/ml. Approximately 96% of strains were susceptible in a rather narrow range between 5 and 10 μg of spectinomycin/ml. The majority of the strains demonstrated identical MIC and MBC values. However, for some strains, especially those susceptible to the lower concentrations, the MIC was lower than the MBC. Even in these instances, the MBC was usually only one dilution higher than the MIC. The concentrations of ampicillin to which the *N. gonorrhoeae* strains were susceptible ranged between 0.06 and 0.5 μg/ml. Approximately 93% of the 113 strains were susceptible to concentrations between 0.12 and 0.25 μg/ml. Also, a number of strains in this group exhibited significant differences between the MIC and MBC values. The susceptibility of *N. gonorrhoeae* to penicillin showed a wide range, from 0.2 to 1.25 units/ml. The largest number of strains (45%) was represented in the MIC group of 0.31 units/ml. This group also showed a discrepancy between the MIC and the MBC.

In Table 2, the MBC of spectinomycin is compared with those of penicillin and ampicillin. We have not listed the one strain for which the MBC of spectinomycin was 2.5 μg/ml. No correlation of the susceptibility of the strains to the three drugs was observed. Regardless of the MBC of spectinomycin, the MBC of ampicillin and penicillin against the same strains showed the full range of variations.

TABLE 2. *Comparison of the MBC of spectinomycin with the MBC of ampicillin and penicillin*

Spectinomycin		Ampicillin		Penicillin	
No. of strains[a]	MBC (μg/ml)	No. of strains	MBC (μg/ml)	No. of strains	MBC (units/ml)
4	20	3	0.25	1	0.63
		1	0.12	1	0.16
				1	0.08
				1	0.04
85	10	3	0.50	1	1.25
		47	0.25	30	0.63
		33	0.12	27	0.31
		2	0.06	18	0.16
				7	0.08
				2	0.02
23	5	1	0.50	4	0.63
		10	0.25	11	0.31
		10	0.12	1	0.16
		2	0.06	4	0.08
				2	0.04
				1	0.02

[a] One strain exhibiting an MBC of 2.5 μg/ml has not been listed.

In Table 3, the MBC of the three drugs against the strains tested is compared with the concentrations of the drugs reported to be attained in serum. After treatment with the doses of antibiotics listed, the concentration in the serum, even after 6 h, exceeds the highest observed bactericidal concentration.

DISCUSSION

The number of cases of gonorrhea is increasing, and reactions to penicillin occur in approximately 5 to 10% of patients receiving this antibiotic. As many as 300 persons die each year in the United States as result of penicillin anaphylaxis. Antibiotics substituting for penicillin in treatment of gonorrhea in such situations are therefore required. The lack of cross-resistance and cross-allergenicity with penicillin make spectinomycin attractive for treatment of gonorrhea, especially in patients in whom resistance to penicillin has developed or in patients who claim to be allergic to penicillin. We have examined the susceptibility of *N. gonorrhoeae* by the broth dilution method to determine both the MIC and the MBC, the latter not being obtainable by the agar dilution method. It seems justifiable that both the MBC and the MIC should be determined for a new drug.

Approximately 10 to 15% of the strains exam-

TABLE 3. *Comparison of the MBC of three antibiotics against N. gonorrhoeae with the concentrations achieved in serum*

Antibiotic[a]	Single dose	Route	Concn in serum[b]					MBC range[b]
			1 h	2 h	4 h	6 h	12 h	
Spectinomycin.....	2.0 g	i.m.	105	95	50	30	5	2.5–20
Ampicillin.....	3.5 g	Oral	5	18	14	3	<1	0.06–0.5
Penicillin.....	3×10^6 units	i.m.	8	4	2.5	1.5	1	0.02–1.25

[a] The serum concentrations of spectinomycin are from technical data supplied by The Upjohn Co.; those of ampicillin and penicillin are those reported by Johnson et al. (1).

[b] Concentrations of spectinomycin and ampicillin are expressed in micrograms per milliliter; those of penicillin are in units per milliliter.

ined showed an MIC that was lower than the MBC. In most cases, the MBC was only one dilution higher. The discordant results suggest that the agar dilution method, which determines the MIC value only, will occasionally give erroneous results. The laborious broth dilution method has, however, some disadvantages: it is more time-consuming than the plate technique; the possibility of contamination, because of multiple manipulations, is greater; and the barely visible growth of *N. gonorrhoeae* in Mueller-Hinton broth makes the reading of the MIC sometimes difficult. Our MIC values of spectinomycin for *N. gonorrhoeae* obtained by the broth dilution method compare favorably with the agar dilution results reported by Pedersen et al. (3) and in technical data supplied by The Upjohn Co.

The data presented on the in vitro susceptibility of *N. gonorrhoeae* to spectinomycin should be of practical value to the clinician. Since this antibiotic is not yet in wide use, it may be assumed that the MIC and MBC values obtained represent the "natural" susceptibility of *N. gonorrhoeae* to this drug, and this susceptibility should, at least for some time, be the same in various parts of this country.

LITERATURE CITED

1. Johnson, D. W., P. A. Kvale, V. L. Afable, S. D. Steward, C. W. Halverson, and K. K. Holmes. 1970. Single-dose antibiotic treatment of asymptomatic gonorrhoea in hospitalized women. N. Engl. J. Med. **283**:1–5.
2. Laird, S. M., and G. Taylor. 1962. Treatment of gonorrhoea with actinospectacin. Brit. J. Vener. Dis. **38**:60–63.
3. Pedersen, A. H. B., P. J. Wiesner, K. K. Holmes, C. J. Johnson, and M. Turck. 1972. Spectinomycin and penicillin G in the treatment of gonorrhoea. J. Amer. Med. Ass. **220**:205–208.
4. Sparling, P. F., A. R. Yobs, T. E. Billings, and J. F. Hackney. 1966. Spectinomycin sulfate and aqueous procaine penicillin G in treatment of female gonorrhoea. Antimicrob. Ag. Chemother. 1965, p. 689–692.
5. Tiedemann, J. H., J. F. Hackney, and E. V. Price. 1965. Acute gonorrheal urethritis in men. Treatment with spectinomycin sulfate. J. Amer. Med. Ass. **191**:89–91.
6. Wilcox, R. R. 1962. Trobicin (actinospectacin), a new injectable antibiotic in the treatment of gonorrhoea. Brit. J. Vener. Dis. **38**:150–153.