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In Vitro Activity of p-Hydroxybenzyl Penicillin (Penicillin X) and Five Other Penicillins Against *Neisseria gonorrhoeae*: Comparisons of Strains from Patients with Uncomplicated Infections and from Women with Pelvic Inflammatory Disease

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Minimum inhibitory concentrations (MICs) of six penicillins against 95 strains of *Neisseria gonorrhoeae* from patients with uncomplicated anogenital infections and 22 strains from women with pelvic inflammatory disease were determined by an agar plate dilution method, using an inocula replicator. Against all 117 strains, the order of activity observed was: BL-P1654 > penicillin X > penicillin G > ampicillin > amoxicillin = carbenicillin. MICs against strains isolated from women with gonococcal pelvic inflammatory disease were significantly higher than those against isolates from uncomplicated infections: BL-P1654, $P < 0.001$; penicillin X, $P < 0.001$; penicillin G, $P < 0.001$; ampicillin, $P < 0.001$; and amoxicillin, $P < 0.05$. MICs of penicillin G were ≥ 0.125 μg/ml against 33 (36%) of the 92 strains from patients with uncomplicated infections, as contrasted with 15 (68%) of the 22 isolates from women with pelvic inflammatory disease ($P < 0.01$). The means of the MICs of penicillin G were 0.06 μg/ml for the former and 0.14 μg/ml for the latter.

Continuous monitoring of the susceptibility of strains of *Neisseria gonorrhoeae* from cases of acute uncomplicated infections in men and women has shown that the activity of penicillin G against such isolates has not changed much over the past five years (9). Thus, in 1974, the Center for Disease Control (CDC) still considered penicillin G the drug of choice for such uncomplicated infections (1). However, strains of gonococci with increased resistance to penicillin G are being encountered (2), and there is a continuing need to find more active antigonococcal agents.

Penicillin X (*p*-hydroxybenzyl penicillin) was identified in 1944 by workers in the Imperial College, London, and at the Northern Regional Research Laboratories in Peoria, Ill. (6). Several groups of workers (4, 7, 13, 14, 20) showed that penicillin X, which, like penicillin G, is susceptible to penicillinase, was two or more times more active than penicillin G against gonococci in vitro. However, Romansky and Robin (17) found the two penicillins equally active. Ory et al. (15) and Welch et al. (20) also demonstrated significantly higher levels of activity in serum after intramuscular doses of penicillin X than after equivalent doses of penicillin G. Subsequently, Meads et al. (13) showed no such advantage when the drugs were taken orally. Welch et al. (20) also showed that penicillin X given intramuscularly was much more effective in treating acute gonorrhea than commercial penicillin (G).

The purpose of the present study was twofold: (i) to compare the activity of penicillin X in vitro with those of five other penicillins against recent isolates of *N. gonorrhoeae* and (ii) to compare the susceptibility of isolates from uncomplicated acute gonococcal infections with that of isolates from women with gonococcal pelvic inflammatory disease (PID).

MATERIALS AND METHODS

Two groups of strains of *N. gonorrhoeae* were studied. One consisted of 22 strains isolated from endocervical cultures from women with gonococcal PID who were treated at Boston City Hospital between 1973 and 1975. The diagnosis of pelvic inflammatory disease was based on the presence of lower abdominal tenderness and/or pain on manipulation of the uterine cervix. The other strains were obtained as part of the National Gonorrhea Therapy Monitoring Study (9, 10). They were isolated from

both men and women who presented at the outpatient clinic of the Boston City Hospital with uncomplicated anogenital gonorrhea during 1973 and 1974. From the latter group, 95 strains were selected on the basis of previous tests with penicillin G to reflect the distribution of minimum inhibitory concentrations (MICs) of penicillin G against all strains isolated at Boston City Hospital (9). For 61% of the selected strains these MICs were ≠0.06 μg/ml, as compared to 65% of all the strains that were isolated at Boston City Hospital.

Presumptive identification of the organisms was made on the basis of the Gram stain and oxidase reaction (*N,N*-dimethyl-*p*-phenylenediamine monohydrochloride). The identity was confirmed by sugar fermentations both in this laboratory and at CDC in Atlanta. The isolates were stored at −80°C in Trypticase soy broth to which 20% glycerol was added. Before each set of tests, the organisms were thawed, inoculated onto chocolatized (5% sheep blood) Mueller-Hinton agar, and incubated in a candle jar for 48 h. Broth cultures were prepared by inoculating two or three colonies from the chocolate agar plate into 5 ml of Mueller-Hinton broth containing 5% chocolatized blood. These broth cultures were incubated for 48 h at 37°C and used undiluted in the tests; they contained approximately 10^9 colony-forming units per ml.

The following penicillins were used in this study: penicillin X sodium, penicillin G sodium, and carbenicillin monohydrate monosodium, provided by Pfizer Pharmaceuticals and its Roerig Division; ampicillin sodium and BL-P1654 sodium, provided by Bristol Laboratories; and amoxicillin trihydrate, provided by Beecham Laboratories. Aqueous solutions of the dried standard powders were prepared to contain 2,000 μg of activity per ml, and samples were stored at −20°C for not more than 2 weeks. The solutions were used on the day they were thawed, and the unused portions were discarded.

Tests for susceptibility to the penicillins were performed by the plate dilution method, using the inocula replicator of Steers et al. (19), which delivers approximately 0.002 ml of each culture. Twofold dilutions of the penicillins in chocolatized agar were prepared over a range from 4.0 to 0.001 μg/ml. The inoculated plates were incubated at 37°C for 48 h in candle jars, after which the plates were independently read for growth by two of us. The highest concentration of antibiotic on which no growth or not more than five colonies were visible with the aid of a hand lens (×3) was taken as the MIC. Controls of *Staphylococcus aureus* 209P and *Streptococcus pyogenes* 98 were included with each test.

The paired-*t* test (8) was used in comparisons of MICs of various penicillins against groups of the same isolates. An unpaired-*t* test was used to compare MICs of penicillins against isolates from uncomplicated cases with those against isolates from cases of PID.

RESULTS

The results of all the tests done with each of the six penicillins are shown in Table 1 (lowest part). The MICs of these antibiotics for the strains isolated from uncomplicated cases are shown separately, in the top portion of the table, and those for the strains from women with PID are shown in the middle part. Due to the relatively small number of the latter, the range and median MICs of each of the penicillins were essentially the same for all strains as those for the strains from the uncomplicated cases. The median MICs of penicillin G, penicillin X, and

TABLE 1. *Susceptibility of N. gonorrhoeae to six penicillins*

Strains	Penicillin	No. of strains with MIC (μg/ml) of:									Total strains
		0.003	0.007	0.015	0.03	0.06	0.125	0.25	0.5	1.0	
From acute uncomplicated cases	Penicillin G	2	14	6	14	*23*[a]	19	11	3		92
	Penicillin X	1	10	13	19	*20*	25	7	0		95
	BL-P1654	2	10	22	*21*	12	18	0	0		85
	Ampicillin			10	14	*29*	28	2	1		84
	Amoxicillin			4	12	15	*34*	17	2		84
	Carbenicillin			2	3	0	0	2	*6*	4	17
From women with PID	Penicillin G			1	1	5	*5*	7	3		22
	Penicillin X			0	2	3	*10*	7	0		22
	BL-P1654			0	2	*4*	3	1	0	1	11
	Ampicillin			0	0	2	*6*	4	0		12
	Amoxicillin			0	0	2	4	*5*	1		12
	Carbenicillin			1	0	2	0	6	*3*	10	22
From all cases	Penicillin G	2	14	7	15	*28*	24	18	6	0	114
	Penicillin X	1	10	13	21	*23*	35	14	0	0	117
	BL-P1654	2	10	22	*23*	16	21	1	0	1	96
	Ampicillin			10	14	*31*	34	6	1	0	96
	Amoxicillin			4	12	17	*38*	22	3	0	96
	Carbenicillin			3	3	2	0	8	*9*	14	39

[a] Median MIC is shown in italics.

ampicillin were the same (0.06 μg/ml); the median MIC of BL-P1654 was one-half of that concentration, whereas it was twofold higher (0.125 μg/ml) for amoxicillin and highest (0.5 μg/ml) for carbenicillin.

The MICs for the strains from patients with PID were in a higher range than those for the strains from uncomplicated cases, and the median MICs were twice as high for the former for all the penicillins except carbenicillin. The MICs of penicillin G, penicillin X, and BL-P1654 for the PID strains were ≥0.03 μg/ml for all but one, whereas one-fourth to two-fifths of those from uncomplicated cases were inhibited by ≤0.015 μg/ml. MICs of ampicillin and amoxicillin for the PID strains were all ≥0.06 μg/ml, whereas for a large proportion of those from uncomplicated cases the MICs were ≤0.03 μg/ml. The MICs of carbenicillin did not show such differences.

Cumulative distribution curves of the MICs of penicillin G and the four other "available" penicillins are shown in Fig. 1 for the strains from uncomplicated cases (bottom) and for those from PID (top). Particularly notable are the higher MICs (shift of the curves to the right) of amoxicillin and, especially, carbenicillin and the bimodal distribution of the MICs of carbenicillin.

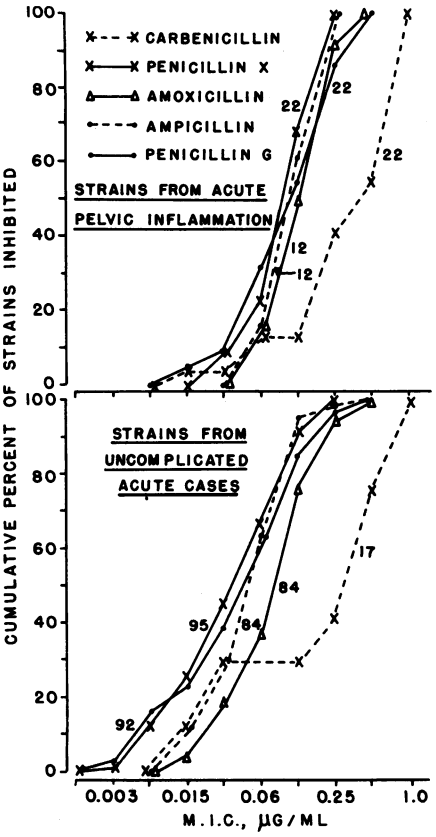

FIG. 1. *Susceptibility of strains of N. gonorrhoeae from uncomplicated acute infections (bottom) and from cases of pelvic inflammation (top) to six penicillins. The number of strains tested is shown for each of the agents.*

Table 2 shows the results of a statistical evaluation, by the paired-*t* test, of the differences in activity of the six penicillins. For all the strains and for those from uncomplicated infections, BL-P1654 was significantly more active than any of the others. Penicillin X was more active than the others except BL-P1654. Penicillin G, in turn, was significantly more active than ampicillin, amoxicillin, and carbenicillin. Ampicillin was more active than amoxicillin, but the activity of the latter was not significantly greater than that of carbenicillin.

The numbers of tests of strains from cases of PID were too few to achieve statistically significant differences in most of the paired-*t* tests. However, penicillin G, penicillin X, and BL-P1654 were significantly more active than amoxicillin and carbenicillin, and penicillin X was also significantly more active than ampicillin for the strains tested with these two analogs.

Table 3 shows direct comparisons of the MICs of five penicillins with those of penicillin G. Penicillin X and penicillin G were equally active against nearly one-half of the strains; the former was more active against about one-third and less active against about one-fifth of the strains. BL-P1654 was more active than penicillin G against a much larger proportion (nearly 60%) of the strains, and less active against a small proportion (16%). Ampicillin, amoxicillin, and carbenicillin compared less favorably with penicillin G.

Table 4 lists the geometric means of the MICs of all strains tested with each of the six antibiotics, comparing the strains from uncomplicated cases with those from patients with pelvic inflammation. The differences are approximately two- to threefold for all of the penicillins and are highly significant for each of the penicillins except carbenicillin.

DISCUSSION

The strains of *N. gonorrhoeae* tested for susceptibility to penicillins in this study were all isolated from 1973 to 1975. Those previously tested in this laboratory with 66 antibacterial agents, including 16 penicillins and 12 cephalosporins as well as spectinomycin, were isolated in 1972 (5). In both of these studies, nearly

TABLE 2. *Comparisons of MICs of six penicillins: probabilities from paired-t test*

Strains	Penicillin	P^a for:				
		Penicillin X	BL-P1654	Ampicillin	Amoxicillin	Carbenicillin
From uncomplicated cases	Penicillin G	0.035+	<0.001+	0.004	<0.001	<0.001
	Penicillin X		<0.001+	<0.001	<0.001	<0.001
	BL-P1654			<0.001	<0.001	0.039
	Ampicillin				<0.001	NS
	Amoxicillin					NS
From cases with PID	Penicillin G	NS	NS	NS	0.025	<0.001
	Penicillin X		NS	0.026	<0.001	<0.001
	BL-P1654			NS	0.012	0.013
	Ampicillin				NS	NS
	Amoxicillin					NS
From all cases	Penicillin G	0.022+	<0.001+	<0.001	<0.001	<0.001
	Penicillin X		<0.001+	<0.001	<0.001	<0.001
	BL-P1654			<0.001	<0.001	0.001
	Ampicillin				<0.001	0.048
	Amoxicillin					NS

[a] *P*, Probability derived from paired-*t* test; NS, not significant, +, penicillin at head of column is more active (i.e., has a lower MIC) than that listed on the left.

TABLE 3. *Susceptibility of strains of N. gonorrhoeae to penicillin X and five other penicillins compared with penicillin G*

Strains	Penicillin	No. of strains with activity (MIC), compared with that of penicillin G (=1), of:									Total
		1/16	1/8	1/4	1/2	1	2	4	8	16	
From acute, uncomplicated cases	BL-P1654	1	1	11	*35*[a]	22	10	2			82
	Penicillin X		1	2	27	*42*	17	3			92
	Ampicillin		1	3	13	*28*	23	8	3		79
	Amoxicillin			3	3	16	*35*	20	4		81
	Carbenicillin				0	3	4	*7*	2		16
From cases with PID	BL-P1654				5	*3*	2	1			11
	Penicillin X				7	*11*	4				22
	Ampicillin				1	*7*	2	2			12
	Amoxicillin				1	4	*5*	2			12
	Carbenicillin					4	*8*	6	2	2	22
From all cases	BL-P1654	1	1	11	*40*	25	12	3			93
	Penicillin X		1	2	34	*53*	21	3			114
	Ampicillin		1	3	14	*35*	25	10	3		91
	Amoxicillin			3	4	20	*40*	22	4		93
	Carbenicillin					7	*12*	13	44	2	38

[a] Median numbers are shown in italics.

all of the strains tested were inhibited by all the β-lactamase-susceptible penicillins in concentrations readily attainable in the serums (<1.0 μg/ml). Carbenicillin in both studies and also ticarcillin in the earlier one were the least active among those penicillins. For methicillin and the other relatively penicillinase-resistant penicillins, the MICs were in a higher range, up to 3.1 to 12.5 μg/ml, and those of the cephalosporins were still higher. These results, in general, justified the conclusion reached in the National Gonorrhea Therapy Monitoring Study that penicillin G was still the agent of choice for initial therapy of uncomplicated gonorrhea (9).

The present study was undertaken, in the first instance, to reexamine the possible place of penicillin X in the therapy of gonorrhea by comparing its activity with that of four other available penicillins and also with BL-P1654, which was of interest because of its greater

TABLE 4. *Comparisons of MICs of six penicillins against strains of N. gonorrhoeae isolated from uncomplicated cases with those from women with PID*

Antibiotic	Strains from uncomplicated cases				Strains from women with PID				P[c]
	No. tested	Mean MIC[a]	SEM[b] +	SEM[b] −	No. tested	Mean MIC	SEM +	SEM −	
Penicillin G	92	0.057	0.058	0.045	22	0.137	0.167	0.113	<0.001
Penicillin X	95	0.047	0.052	0.042	22	0.125	0.143	0.109	<0.001
BL-P1654	85	0.031	0.035	0.028	11	0.097	0.131	0.072	<0.001
Ampicillin	84	0.063	0.068	0.058	12	0.140	0.162	0.122	<0.001
Amoxicillin	84	0.098	0.107	0.089	12	0.167	0.200	0.139	0.028
Carbenicillin	17	0.221	0.323	0.152	22	0.401	0.511	0.315	0.242

[a] Geometric mean of the MICs in micrograms per milliliter.

[b] SEM, Standard error of the mean.

[c] *P* values were derived by the unpaired-*t* test (6), except for that of carbenicillin, which was derived from the Wilcoxon two-sample, rank sum test (18).

activity (5) although it was not being considered for clinical use. The data indicated that, quantitatively, penicillin X was indeed "significantly" more active than penicillin G and the other penicillins except BL-P1654 against the strains from uncomplicated gonorrhea. The descending order of activity of the six analogs was: BL-P1654, penicillin X, penicillin G, ampicillin, amoxicillin, and carbenicillin. However, although the differences between the MICs of successive pairs of penicillins in this order were "statistically significant" (except between amoxicillin and carbenicillin), the differences between them were not of a magnitude to be of equal "clinical importance."

During 1976 there appeared reports of strains isolated from various parts of the United States, Canada, several European countries, and, particularly, countries in eastern Asia that had much greater resistance to penicillin, with MICs much behond those achievable in vivo even with massive doses (2, 3). These strains were shown to produce β-lactamase (penicillinase), presumed to be plasmid-mediated, and, in one report (16), also found to be resistant to cephaloridine but not to another cephalosporin, cefuroxime, currently under investigation. Because the studies reported here, as well as the earlier ones in this laboratory, were all done with inocula of undiluted cultures in the plate dilution, replica inoculator method, it is unlikely that any of the strains included in these studies were β-lactamase producers. Currently, spectinomycin appears to be the drug of choice for treating gonorrhea when penicillins and other effective agents fail, but it too has its limitations (12), although penicillin is still recommended as the initial drug of choice in acute uncomplicated cases (2).

The second purpose of the present study was to compare the susceptibility of strains of *N. gonorrhoeae* isolated from women with PID with that of strains from patients with uncomplicated acute infections. MICs against the former were shown to be significantly higher than those required to inhibit the latter. The average difference was about twofold or more for each of the penicillins except amoxicillin and carbenicillin, as shown in Table 4.

It is now well established that strains of *N. gonorrhoeae* from patients with disseminated gonococcal infection differ from strains isolated from patients with uncomplicated anogenital infections in that they are more susceptible to penicillin and other antibacterial agents (21) and have unique auxotrophic growth requirements (11). This is in contrast to the data presented here, which show that strains from patients with PID are more resistant to penicillins than their contemporaneous isolates from uncomplicated anogenital infections. This suggests that the organisms that cause PID, an important complication of gonococcal infection, may also have unique biological characteristics.

This paper deals only with the results of in vitro tests. However, factors other than susceptibility in vitro may be of equal or even greater or more crucial importance in the choice of a drug for therapy. These include patient acceptability, toxicity, and pharmacokinetics, including absorption and penetration into body fluids and compartments, or into cells, or through the fibrinous or fibrous surroundings of localized areas of suppuration. Thus, penicillin X had been shown to provide higher levels of activity than penicillin G in equal doses given intramuscularly (15) but not when taken orally (13). Other penicillins and other antibacterial agents, particularly new ones, that appear promising from their in vitro activity will have to be more fully explored in other respects be-

fore their relative value in the therapy of acute uncomplicated gonorrhea and other focal or disseminated gonococcal infections can be fully assessed.

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