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Comparative In Vitro Activities of Pristinamycin and Other Antimicrobial Agents against Genital Pathogens

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The MICs of pristinamycin for genital pathogens were compared with those of ampicillin, tetracycline, erythromycin, and ciprofloxacin. Pristinamycin was active against all the strains studied. Because of this activity and its lack of toxicity, pristinamycin might be a valuable therapeutic agent for treating major sexually transmitted diseases.

Most bacterial sexually transmitted diseases can be effectively treated. However, the emergence and spread of plasmid- or chromosomally mediated antimicrobial resistance to penicillins, tetracyclines, and erythromycins limit the use of these antibiotics. Since sexually transmitted diseases caused by different pathogens are frequently concurrent, especially in women (6), treatment with a single antibiotic with a broad spectrum of activity against these pathogens would be of great clinical value. Pristinamycin, a natural antibiotic which belongs to the family streptogramins and is composed of several compounds, shares the antimicrobial-activity spectra of macrolides and lincosamides (2, 8). However, fewer strains are resistant to pristinamycin (8, 9) than to macrolides and lincosamides. This antibiotic has been shown to have high in vitro activity against gonococci, including penicillinase-producing strains (11, 13) and mycoplasmas (10). There are no data available about its activity against *Chlamydia trachomatis* and *Gardnerella vaginalis*. We have therefore examined the in vitro activities of pristinamycin against clinical isolates of *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *C. trachomatis*, and *G. vaginalis*.

A total of 15 penicillinase-producing *N. gonorrhoeae*, 50 non-penicillinase-producing *N. gonorrhoeae*, 40 *M. hominis*, 30 *U. urealyticum*, 12 *C. trachomatis*, and 50 *G. vaginalis* strains were used. All the strains were isolated from patients with genital diseases who were attending the Sexually Transmitted Diseases Clinic of the Purpan Hospital of Toulouse (1986 to 1988). The organisms were stored at −70°C until use.

The antimicrobial agents used were pristinamycin (Specia), ampicillin (Bristol Laboratories), tetracycline (Diamant), erythromycin (Abbott Laboratories), and ciprofloxacin (Bayer).

MIC tests with *N. gonorrhoeae* and *G. vaginalis* were performed by an agar dilution technique with Mueller-Hinton agar supplemented with 5% horse blood, as described previously (7). Twofold serial concentrations from 0.001 to 4 μg/ml were incorporated into the medium for *N. gonorrhoeae* tests; doubling concentrations of 0.004 to 128 μg/ml were used for *G. vaginalis* tests. The inocula were prepared from a 24-h culture on agar medium and diluted to obtain 10^5 CFU per spot. After incubation of the inocula for 48 h in 10% CO_2 at 37°C, the MIC was determined by observing the lowest concentration of antibiotic in which bacterial growth was inhibited. In each experiment, World Health Organization *N. gonorrhoeae* reference strains A, B, C, D, and E were included; the MICs of tetracycline and pristinamycin for these strains were similar to those obtained by Riou et al. with peptone agar (11).

MIC testing of *M. hominis* was performed by an agar dilution technique with modified Hayflick medium without penicillin. Plates containing serial dilutions of antibiotics (0.06 to 64 μg/ml) were inoculated with 10^4 CFU/ml by using a multipoint inoculator. The plates were then incubated at 37°C in 5% CO_2 for 48 h and read under a stereomicroscope. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited the development of visible growth on agar plates. In each experiment, two reference strains, *Staphylococcus aureus* ATCC 25923 and *M. hominis* PG21, were included. The MICs for the *S. aureus* strain were established on Mueller-Hinton agar and compared with those obtained with Hayflick medium. The MIC of tetracycline for strain PG21 was 64 μg/ml.

The MICs for *U. urealyticum* were determined in microdilution plates (12 by 8 wells). The growth medium was Shepard broth without penicillin. The plates containing serial dilutions of antibiotics (0.06 to 64 μg/ml) were inoculated with 10^4 CFU/ml and incubated in 5% CO_2 for 48 h. The MIC was defined as the lowest concentration of antimicrobial agent which prevented color change. In each experiment, *S. aureus* ATCC 25923 and one laboratory strain, *U. urealyticum* 2K160, kindly supplied by C. Bonnissol, Institut Pasteur de Paris, were used as controls. The MICs for the *S. aureus* strain were determined on Mueller-Hinton agar and compared with those obtained with Shepard broth. The MIC of tetracycline for strain 2K160 was 0.5 μg/ml.

The MIC for *C. trachomatis* was determined by a modification of a previously described method (12). All the clinical isolates and a laboratory strain of *C. trachomatis* (LGV-II ATCC VR-902) used as a control were passaged extensively in the laboratory and at least twice in antibiotic-free maintenance medium (Eagle minimum essential medium with 10% fetal bovine serum, 1% glutamine, and 5 g of glucose per liter). Monolayers of antibiotic-free McCoy cells were grown in 96-well microdilution plates seeded at a concentration of 2.0×10^5 cells per ml. After 48 h of incubation, the *C. trachomatis* strains were each inoculated (250 to 500 inclusion-forming units per well). Monolayers were then centrifuged for 60 min at 1,500 × *g*, aspirated, and overlaid with appropriate serial dilutions of the antibiotic being tested (0.03 to 64 μg/ml). All dilutions were made with the above-

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TABLE 1. Comparative activities of pristinamycin against genital pathogens

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
N. gonorrhoeae, non-penicillinase producing (50)	Pristinamycin	0.016–0.5	0.06	0.25
	Ampicillin	0.016–1	0.06	0.5
	Tetracycline	0.125–4	0.5	2
	Erythromycin	0.03–1	0.125	0.5
	Ciprofloxacin	0.002–1	0.004	0.008
N. gonorrhoeae, penicillinase producing (15)	Pristinamycin	0.03–0.5	0.125	0.5
	Ampicillin	>2	>2	>2
	Tetracycline	0.5–4	1	2
	Erythromycin	0.06–0.5	0.125	0.5
	Ciprofloxacin	0.002–1	0.004	0.008
C. trachomatis (12)	Pristinamycin	0.5	0.5	0.5
	Ampicillin	>32	>32	>32
	Tetracycline	0.06	0.06	0.06
	Erythromycin	0.06–0.125	0.06	0.06
	Ciprofloxacin	1	1	1
U. urealyticum (30)	Pristinamycin	0.25–2	1	2
	Tetracycline	0.25–32	1	4
	Erythromycin	0.5–16	2	8
	Ciprofloxacin	2–32	4	8
M. hominis (40)	Pristinamycin	0.5–2	1	1
	Tetracycline	0.25–64	0.5	8
	Erythromycin	>32	>32	>32
	Ciprofloxacin	0.5–2	1	2
G. vaginalis (50)	Pristinamycin	0.016–0.125	0.06	0.125
	Ampicillin	0.06–1	0.125	0.5
	Tetracycline	0.125–128	2	64
	Erythromycin	0.008–0.125	0.03	0.06
	Ciprofloxacin	0.5–16	1	2

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

mentioned antibiotic-free maintenance medium containing 0.5 μg of cycloheximide per ml. Appropriate antibiotic-free controls were included on each plate. Cultures were incubated for 48 h at 35°C and then fixed and stained for enumeration of inclusions by using a fluorescein-conjugated monoclonal antibody (Ortho Diagnostics, Inc.). The lowest concentration of each antibiotic which completely inhibited inclusion formation was defined as the MIC. In each experiment, the reference strain (LGV-II) was included; the MIC of tetracycline for this strain was 0.06 μg/ml.

The MICs of pristinamycin and the other antimicrobial agents for isolates of *N. gonorrhoeae*, *C. trachomatis*, *U. urealyticum*, *M. hominis*, and *G. vaginalis* are shown in Table 1. The break point for pristinamycin, calculated on the basis of standards established by the Comité Français de l'Antibiogramme (3), is ≤2 μg/ml. All the strains studied were susceptible, according to this break point. Pristinamycin had a high intrinsic activity against each strain of *N. gonorrhoeae* tested. No difference was observed between penicillinase- and non-penicillinase-producing strains. Our results are in agreement with those previously reported (11, 13). *C. trachomatis* (MIC, 0.5 μg/ml) appeared to be less susceptible to pristinamycin than gonococci. Pristinamycin was less active than tetracycline and erythromycin against this organism but more active than ciprofloxacin or ampicillin. These findings confirm the possibility that pristinamycin could be of benefit in the therapy of chlamydial infections. This possibility was suggested by a study in which 40 patients who were given 500 mg of pristinamycin four times daily for 2 weeks were cured of nongonococcal urethritis (1). The tetracyclines and erythromycin are the drugs most frequently used to treat infections associated with mycoplasmas. These compounds are not ideal, because of the spread of *tetM*-mediated resistance and (in the case of erythromycin) lack of activity against *M. hominis*. Pristinamycin with ciprofloxacin was the only compound used in this series which demonstrated potentially useful activity against mycoplasmas. Our results were similar to those reported by Quentin et al. (10). Furthermore, for mixed infections involving *M. hominis* (bacterial vaginosis and pelvic inflammatory disease), pristinamycin appears very promising. This antibiotic was reported to show in vitro activity similar to that of chloramphenicol against anaerobic bacteria (5). It showed excellent activity against *G. vaginalis* in our series, with a MIC for 90% of strains tested of 0.125 μg/ml. Although discrepancies have been found between the in vitro and in vivo susceptibilities of *G. vaginalis*, clinical studies of pristinamycin in bacterial vaginosis appeared justified.

A number of problems compromise the efficacy of currently available regimens in the antimicrobial therapy of bacterial sexually transmitted diseases. These include antimicrobial resistance, frequent side effects, lack of alternative regimens for pregnant women or patients with drug allergies, and ineffectiveness of most regimens against all pathogens in polymicrobial infections or in simultaneously occurring infections. Pristinamycin is an especially well-tolerated antibiotic without toxic side effects (4, 9). The results of our in vitro study show its broad spectrum of activity against major genital pathogens. In preliminary trials, the drug appears effective in the treatment of gonococcal and nongonococcal urethritis (1); studies of its clinical effectiveness in other genital tract infections are warranted.

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