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In Vitro Activity of the Two New 4-Quinolones A56619 and A56620 against *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum* and *Gardnerella vaginalis*

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The in vitro activity of tetracycline, ciprofloxacin and two recently developed 1-aryl-fluoroquinolones, A56610 and A56620, was tested against 65 beta-lactamase-negative and 35 beta-lactamase-positive *Neisseria gonorrhoeae* strains, 12 *Chlamydia trachomatis*, 50 *Mycoplasma hominis*, 28 *Ureaplasma urealyticum* and 50 *Gardnerella vaginalis* strains. In the case of *Chlamydia trachomatis* and *Mycoplasma hominis* both the MIC and the MBC were determined. The MIC90 of ciprofloxacin for *Neisseria gonorrhoeae* was 0.008 μg/ml and of A56619 and A56620 ⩽ 0.03 μg/ml. No difference was observed between the activity against beta-lactamase-negative and beta-lactamase-positive strains. The MIC90 values of ciprofloxacin and A56620 for *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* were identical, the values being 2 μg/ml, 1 μg/ml and 4 μg/ml respectively. The MIC90 of A56619 for *Chlamydia trachomatis* and *Ureaplasma urealyticum* was 0.5 μg/ml and 1 μg/ml respectively. The MBC90 values of the three quinolones for *Chlamydia trachomatis* and *Mycoplasma hominis* were ⩽ 2 μg/ml. The activity of the quinolones against *Gardnerella vaginalis* was rather low, the MIC90 being ⩾ 4 μg/ml. It is concluded that A56619 and A56620 might be useful for single-dose therapy of gonococcal infections.

Ciprofloxacin, a recently developed 4-quinolone, has been reported to have very high activity against *Neisseria gonorrhoeae* and moderate activity against *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* (1, 2, 3). Since the quinolones might be of value in the chemotherapy of sexually transmitted diseases, the in vitro activity of ciprofloxacin, two new 1-aryl-fluorquinolones, A56619 and A56620, and tetracycline was tested against clinical isolates of *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum* and *Gardnerella vaginalis*. In the case of *Chlamydia trachomatis* and *Mycoplasma hominis* both the MIC and MBC were determined.

Materials and Methods

Antibiotics. Ciprofloxacin was obtained from Bayer, FRG, A56619 and A56620 from Abbott, USA, and tetracycline. HCl from Nogepha, The Netherlands.

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Organisms. The 35 beta-lactamase-positive and 65 beta-lactamase-negative *Neisseria gonorrhoeae* strains, 12 *Chlamydia trachomatis*, 50 *Mycoplasma hominis* and 28 *Ureaplasma urealyticum* strains used had recently been isolated from specimens obtained from patients attending the venereological outpatient department of the University Hospital, Rotterdam, The Netherlands. Fifty clinical isolates of *Gardnerella vaginalis* were obtained from the Institute of Tropical Medicine, Antwerp, Belgium.

MIC and MBC Determinations

Neisseria gonorrhoeae. The activity of penicillin G (Gist-Brocades, The Netherlands) and cefuroxime (Glaxo, UK) against this organism was also tested. Twofold dilutions of antibiotics in water were added to a medium consisting of GC medium base (Difco, USA), hemoglobin (Difco, USA) and Isovitalex (BBL, USA). An antibiotic-free control medium was included in each series. The organisms to be tested were cultured overnight. Colonies were freshly suspended in trypticase soy broth (BBL) until an optical density of 0.1 was reached, resulting in an inoculum of 10^6 CFU/ml. These suspensions were inoculated with a Steers multipoint replicator onto the plate series, resulting in spot inocula of 10^4 CFU. All strains were tested for beta-lactamase production by the chromogenic cephalosporin test (Nitrocephin, BBL, USA). After incubation for 18–20 h in a CO_2 incubator (5%) at 37 °C the MIC was determined by observing the lowest concentration of antibiotics in which bacterial growth was completely or almost completely inhibited, as judged by the naked eye. A haze of growth or a single colony was disregarded.

Chlamydia trachomatis. Clinical isolates were passed on a HeLa 229 monolayer seven to nine times in order to increase

the inoculum. Hundred µl of a test train, containing two to nine inclusions per field (magnification x 320), was grown in a 96-well microtiter tissue culture plate (Falcon 3070, Becton Dickinson, USA). After centrifugation at 3000×g for 1 h the inoculum was replaced by 100 µl of growth medium consisting of Eagle's modified Minimal Essential Medium (Flow, UK) with 10% fetal calf serum and 4.5 mM glucose. Twofold dilutions of antibiotics were prepared in growth medium. After 48 h of incubation the monolayers were fixed and subsequently stained with fluorescing monoclonal antibodies (Syva, USA). The plate was turned upside down and the monolayers were screened for the presence of inclusions with a Leitz fluorescence microscope equipped with a long distance objective (32x). One row of wells contained growth medium with the maximal concentration of each antibiotic but no chlamydia in order to detect a cytotoxic effect. No antibiotic was found to be cytotoxic at the maximal concentration tested. Another row of wells contained chlamydia without antibiotics. Every dilution was tested in duplicate. The lowest concentration of antibiotics in which no inclusions could be observed was defined as the MIC. A duplicate plate was used for the subpassage. The monolayer was disrupted by a freeze-thaw procedure. After the second subpassage growth medium was added with 25 µg/ml gentamicin, 25 µg/ml vancomycin and 25 U/ml nystatin. Six subpassages were carried out to determine the MBC. The MBC was defined as the lowest concentration of antibiotics in which no inclusions could be observed after the sixth subpassage.

Mycoplasma hominis. The MIC determinations were carried out in 12 × 8 well microtiter plates. The growth medium consisted of PPLO broth (Difco, USA) as described by Chanock (4), containing 0.05% thallium acetate and 0.2% arginine HCl. Twofold dilutions of antibiotics were prepared in growth medium. One well contained 200 µl of growth medium and 50 µl of *Mycoplasma hominis* suspension, resulting in an inoculum size of 3×10^4 to 1×10^5 CFU/ml. After inoculation the trays were carefully shaken for 30 min in a Titertek mixer and subsequently incubated for three days in a CO_2 incubator (5%) at 35 °C. For the MBC determination 1 µl from each well was inoculated on PPLO agar of the same composition as described above but without thallium acetate. MICs were read after adding one to three drops of 0.0025% phenol red solution to each well. The lowest concentration in which no red-purple colour developed was defined as the MIC. The MBC was defined as the lowest concentration in which no surviving organism could be detected, i.e. the lowest concentration causing at least 99% reduction of the inoculum in three days.

Ureaplasma urealyticum. Clinical isolates of *Ureaplasma urealyticum* were cultured on medium 1 consisting of 23.25 g tryptic soy broth (Oxoid, UK), 20 ml of 1% $MnSO_4.4H_2O$, 110 ml yeast extract as described by Hers (5), 200 ml inactivated horse serum, 5 nk if 20% urea, 2.5 ml of 4% L-cysteine, 16 g agar and 700 ml distilled water. Isolates were transferred to medium 2 consisting of 22.5 g tryptic soy broth, 12.5 ml of 0.2% phenol red, 30 ml yeast extract, 200 ml inactivated horse serum, 50 ml of 20% urea and 750 ml distilled water. The isolates were kept alive by daily transfer in medium 2. As inocula for the MIC determinations, undiluted 18 h cultures in medium 2 were used. Twofold dilutions of the antibiotics were prepared in medium 1 and poured into petri dishes. Multipoint inoculation was carried out using a Steers replicator. Growth was visible on microscopic examination as tiny black colonies after 48 h of incubation under anaerobic conditions (95% N_2 + 5% CO_2) at 35 °C. MICs were also determined on medium 2 with 8.5 g of agar. Growth was indicated by a colour change from orange to purple after 30 h of incubation at 35 °C. The MICs determined by the two methods were virtually identical. The maximal difference was at most one dilution step.

Gardnerella vaginalis. The methods used were similar to those for *Neisseria gonorrhoeae.* Twofold dilutions of antibiotics were prepared in a medium consisting of Mueller-Hinton agar No. 2 (BBL, USA) with 5% fetal calf serum. The plates were incubated at 35 °C in a CO_2 incubator (10%).

Results

No difference was observed between beta-lactamase-positive and beta-lactamase-negative *Neisseria gonorrhoeae* strains as regards susceptibility to the three quinolones tested. Ciprofloxacin displayed the highest activity, with an MIC90 of 0.008 µg/ml. The two new quinolones A56619 and A56620 showed similar activity, with MIC90 values $\leqslant$ 0.03 µg/ml. For beta-lactamase-negative gonococci the MIC50 and MIC90 of penicillin G was 0.08 µg/ml and 1.28 µg/ml respectively. For 2 of 35 beta-lactamase-positive strains and 10 of 65 beta-lactamase-negative strains the MIC of tetracycline was $\geqslant$ 4 µg/ml (Table 1). *Chlamydia trachomatis* appeared to be less susceptible to the quinolones than gonococci, MIC90 values being $\leqslant$ 1 µg/ml.

The lowest MIC90 value of the three quinolones tested, 0.5 µg/ml, was found in the case of A56619. The MBC90 values of the three quinolones were identical (2 µg/ml, Table 2). For almost all *Mycoplasma hominis* strains the MIC and MBC values were $\leqslant$ 1 µg/ml and $\leqslant$ 2 µg/ml respectively. The MIC and MBC of ciprofloxacin for one strain was 2 µg/ml. For two strains the MBC of A56620 was $>$ 2 µg/ml, and for two others ciprofloxacin had the same value (Table 2). For 9 of 49 strains the MBC of tetracycline was $>$ 2 µg/ml. The MIC of tetracycline for two strains and the MBC for another strain was not determined.

The MIC50 and MIC90 values of all the agents tested except A56619 were two to four times higher for *Ureaplasma urealyticum* than for *Mycoplasma hominis.* The MIC of tetracycline was not evaluated for two *Ureaplasma urealyticum* strains.

In general, *Gardnerella vaginalis* demonstrated lower susceptibility than the other micro-organisms to the four agents tested. The MIC90 values of tetracyline, ciprofloxacin, A56619 and A56620 were $\geqslant$ 32, 4, 8, and 4 µg/ml respectively (Table 1).

Discussion

Our study confirms a previous study in which high activity of ciprofloxacin against both beta-lactamase-positive and beta-lactamase-negative gonococci was found (2). In that study an MIC90 of 0.002 µg/ml was reported, which is lower than our value of 0.008 µg/ml. The activity of penicillin G against the

Table 1: MIC values of several antibiotics against *Neisseria gonorrhoea, Ureaplasma urealyticum* and *Gardnerella vaginalis.*

Organisms (n)	Agent	MIC (μg/ml) Range	MIC50	MIC90
Beta-lactamase-positive *Neisseria gonorrhoeae* (35)	tetracycline	0.5–4	1	2
	cefuroxime	≤0.015–1	0.12	0.5
	ciprofloxacin	0.002–0.015	0.004	0.008
	A56619	0.004–0.06	0.008	0.015
	A56620	0.004–0.015	0.004	0.015
Beta-lactamase-negative *Neisseria gonorrhoeae* (65)	tetracycline	0.12–4	0.5	2
	cefuroxime	≤ 0.01–1	0.03	0.25
	penicillin G	0.01–2.56	0.08	1.28
	ciprofloxacin	0.002–0.015	0.004	0.008
	A56619	0.004–0.06	0.015	0.03
	A56620	≤0.001–0.03	0.008	0.015
Ureaplasma urealyticum (28)	tetracycline	0.25–≥16	1	2
	ciprofloxacin	1–4	2	4
	A56619	0.5–1	1	1
	A56620	1–8	4	4
Gardnerella vaginalis (12)	tetracycline	0.25–≥32	8	≥32
	ciprofloxacin	1–4	4	4
	A56619	1–8	8	8
	A56620	1–8	4	4

strains tested was similar. These findings suggest that ciprofloxacin could be of benefit in the therapy of gonococcal infections (6, 7, 8). This was recently confirmed in a study in 57 men who were cured of gonorrhoea after a single oral dose of 250 mg ciprofloxacin (9). In the present study the new 1-aryl-fluoroquinolones, A56619 and A56620, were also found to display high activity against *Neisseria gonorrhoeae* in vitro. Although human pharmacokinetic data on these quinolones have not yet been published, preliminary reports suggest that susceptible micro-organisms should include those for which MIC values are ≤ 4 μg/ml for A56619 and ≤ 2 μg/ml for A56620 (10). In such cases effective therapy of gonococcal infections might be expected.

The activity of ciprofloxacin against *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* was evaluated in previous studies and MIC values of ≤ 2 μg/ml, ≤ 0.5 μg/ml, and 2 μg/ml respectively were reported (1, 3). Our study showed similar results. A56619 was found to be more active against *Ureaplasma urealyticum* and *Chlamydia trachomatis* than the other quinolones.

Chlamydia trachomatis was susceptible to tetracycline, mean serum levels after current therapeutical dosages being 2–3 μg/ml. However, a relatively high MBC (2 μg/ml) was noted for one isolate. This may have been a slow-growing strain which was inhibited incompletely during the short incubation period (48h)

Table 2: MIC and MBC values of four antibiotics against *Chlamydia trachomatis* and *Mycoplasma hominis.*

Organism (n)	Agent	MIC (μg/ml) Range	MIC50	MIC90	MBC (μg/ml) Range	MBC50	MIC90
Chlamydia trachomatis (12)	tetracycline	0.03–0.25	0.06	0.25	0.03–2	0.125	0.25
	ciprofloxacin	0.125–2	0.25	1	0.25–4	1	2
	A56619	0.06–0.5	0.25	0.5	0.125–2	0.5	2
	A56620	0.125–2	0.5	1	0.125–2	0.5	2
Mycoplasma hominis (50)	tetracycline	0.25–1	0.5	1	0.5–>4	1	>4
	ciprofloxacon	0.5–1	1	1	1–>2	2	2
	A56619	0.25–1	0.5	1	0.5–1	1	1
	A56620	0.5–1	1	1	1–>2	2	2

in a medium containing tetracycline, although the possibility of cross contamination cannot definitely be ruled out. For 9 of 49 *Mycoplasma hominis* strains the MBC was > 2 µg/ml. Tetracycline resistance of *Mycoplasma hominis* has been reported previously. A tetracycline MIC $\geqslant 16$ µg/ml was observed for 27 (34%) of 79 isolates (11).

The clinical efficacy of the quinolones in therapy of genital infections caused by *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* has still to be determined. A single oral dose of 250 mg of ciprofloxacin has been shown to be insufficient in therapy of *Chlamydia trachomatis* infections, however single-dose therapy has never proved effective in eliminating this organism (9). Serum levels $\geqslant 2$ µg/ml after well-tolerated oral ciprofloxacin dosages have been reported, but less is known about the tissue distribution of the drug in the urogenital tract (6, 7). Moreover, the interaction of the bacteria in the genital tract remains to be elucidated. After tetracycline therapy *Mycoplasma hominis* but not *Ureaplasma urealyticum* appeared to be eradicated, despite similarly low MICs of tetracyline (12). The low activity of the quinolones against *Gardnerella vaginalis* does not necessarily mean poor efficacy in treatment of bacterial vaginosis, as this condition is mainly the result of anaerobic overgrowth (13). Metronidazole, which is moderately active against *Gardnerella vaginals* in vitro, is very useful in treating cases of vaginosis.

In summary, our study shows that A56619 and A56620 have high in vitro activity against gonococci. A beneficial therapeutic effect might thus be expected after a single oral dose in the case of gonococcal infections. As the in vitro activity of these agents against *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* is lower, higher dosages and prolonged therapy might be required in infections with these organisms.

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