Comparison of the In Vitro Activities of Ofloxacin and Tetracycline Against *Chlamydia trachomatis* as Assessed by Indirect Immunofluorescence

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The in vitro activity of ofloxacin (DL8280), a new pyridone carboxylic acid, against *Chlamydia trachomatis* serotype E was assessed by growth of chlamydia in cycloheximide-treated McCoy cells. Chlamydia-infected monolayers were exposed to the drug for 48 and 96 h and for 48 h followed by 48 h of further incubation after withdrawal of ofloxacin. Inclusions were visualized by indirect immunofluorescence, and the effect of the drug was assessed both by inclusion development and by the number of inclusion-forming units generated in the monolayers. The action of ofloxacin was completely lethal when monolayers were exposed to a concentration of 1 µg/ml throughout one chlamydial developmental cycle. In contrast, tetracycline, when examined by similar techniques, inhibited inclusion development at a concentration of 0.3 µg/ml, but was only lethal at 2.4 µg/ml.

*Chlamydia trachomatis* is now recognized as an important human pathogen, as it is responsible for trachoma, lymphogranuloma venereum, and sexually transmitted infections of the lower genital tract. The latter infections, which are exceedingly prevalent in western societies today, may give rise to serious complications, in particular salpingitis and endometritis in women (16), epididymitis in men (7), and pneumonitis in babies (2). Existing methods to determine the in vitro antichlamydial activity of drugs involve growth of *C. trachomatis* in cultured monolayers in the presence of the drug and assessment of chlamydial development by staining the monolayers with iodine (light microscopy) or Giemsa (dark-ground microscopy) (1, 3, 5, 6, 9, 15). These techniques detect mature *C. trachomatis* inclusions, which contain predominantly elementary bodies. They do not detect the immature inclusions that are found early in the cycle and only contain reticulate bodies; neither do they reliably detect the abnormal inclusions that are induced by certain drugs and contain aberrant chlamydial forms that revert to normal chlamydial development if the drug is removed. Therefore, serial passage after withdrawal of the drug is necessary to distinguish those drugs which merely suppress chlamydial development (e.g., β-lactams) from those which eradicate the infection (e.g., rifampin).

In contrast to iodine and Giemsa (dark-ground microscopy), chlamydiae can be detected by immunofluorescence (IF) at all stages of their intracytoplasmic developmental cycle (13), and IF has proved to be more sensitive than Giemsa at detecting mature inclusions in cycloheximide-treated McCoy cells (14). This report describes a new method of testing drugs for in vitro antichlamydial activity by indirect IF, which is simpler but equally as sensitive as previously described techniques. In this test, ofloxacin, a new pyridone carboxylic acid, was lethal for *C. trachomatis* at a concentration of 1 µg/ml. This compared favorably with tetracycline, which was inhibitory at 0.3 µg/ml but only lethal at concentrations of 2.4 µg/ml or greater.

**MATERIALS AND METHODS**

McCoy cells. McCoy cells were grown in antibiotic-free medium which consisted of Eagle minimal essential medium (Flow Laboratories, Inc., Rockville, Md.), supplemented with 2 mM glutamine–4.4% (wt/vol) sodium bicarbonate–10% (vol/vol) fetal bovine serum.

*Chlamydial strain.* *C. trachomatis* serotype E (strain T181) (12) was used throughout this study. Organisms which had already been passed 10 times in McCoy cells were passaged twice in monolayers grown in antibiotic-free medium. Stocks of cell culture harvest 12 were stored in aliquots in 10% (wt/vol) sorbitol, and a standard inoculum of this material which yielded approximately 10⁵ inclusion-forming units per monolayer was used in each experiment.

**Growth of C. trachomatis.** McCoy cell monolayers were prepared in 1 ml of growth medium on circular glass cover slips (diameter, 10 mm) in flat-bottomed plastic vials. *C. trachomatis* was inoculated into the growth medium of confluent monolayers, and cultures were centrifuged at 2,000 × g for 1/2 h at 35°C. The medium was then replaced with 1 ml of maintenance medium. This was identical to the growth medium, except that it contained 3% (vol/vol) fetal bovine serum, cycloheximide (1 µg/ml), and appropriate dilutions of the antibiotic being tested (see below). Cultures were incubated for 48 or 96 h at 35°C (for detailed schedules, see below) and then fixed in methanol. Inclusions were stained by indirect IF with a polyclonal rabbit serum raised against the homologous chlamydial strain (8) and a fluorescein-conjugated sheep anti-rabbit serum (Wellcome Diagnostics, Dartford, U.K.). Preparations were examined by fluorescence microscopy, and inclusion development was assessed both qualitatively and quantitatively. The number of inclusions per monolayer was calculated by extrapolation after the number of inclusions in five standard fields per cover slip was counted, examined at ×100 magnification. When there were fewer than 100 inclusions per monolayer, the whole cover slip was scanned at a magnification of ×250 or ×400 (depending on the size of the inclusions), and all inclusions were counted.

**Antibiotics.** Stock solutions (1 mg/ml) of both ofloxacin (Hoechst, Frankfurt, Federal Republic of Germany) and tetracycline hydrochloride (Sigma Chemical Co., St. Louis, Germany) were prepared by dissolving the drug in dimethyl sulfoxide (DMSO) and ethanol (1:1). Ofloxacin was added to the growth medium to a concentration of 10 µg/ml. At this concentration, ofloxacin was shown to be very inhibitory to chlamydial growth (15). Tetracycline and doxycycline were added to the growth medium to a concentration of 10 µg/ml.

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McCoy fresh defined as to assess inclusion of infectivity. Incubation monolayers. In schedule c, the antibiotic-containing medium, and the number of inclusions per monolayer was determined. Hence, the number of inclusion-forming units in the original culture was estimated. In every experiment, two cultures were used for each dilution of drug in each schedule. One monolayer was used to assess inclusion development, and the other was used to assess infectivity. Each experiment was repeated at least three times.

MIC and minimum lethal concentration. The MIC was defined as the lowest dilution of the drug which inhibited chlamydial inclusion development in schedule a. The minimum lethal concentration (MLC) was defined as the lowest dilution of the drug which both inhibited all inclusion development in schedule c and also destroyed all infectivity in schedule c.

RESULTS

The effect of ofloxacin on inclusion development and the infectivity of the chlamydia-infected monolayer is shown in Fig. 1. In all schedules, inclusions were eliminated at an ofloxacin concentration of 1.0 μg/ml (Fig. 1A), and no infectivity was recovered from any of the monolayers exposed to 1 μg/ml (Fig. 1B). The action of ofloxacin on C. trachomatis, therefore, appeared to be entirely lethal, since the MIC and MLC were identical. Ofloxacin concentrations below 0.25 μg/ml had little effect on either inclusion development or infectivity; at 0.25 and 0.5 μg/ml inclusions were fewer, smaller, and less densely stained than in untreated cells, and the infectivity of the monolayers was reduced.

In contrast to ofloxacin, the effect of low (<2 μg/ml) concentrations of tetracycline on C. trachomatis was inhibitory rather than lethal. No inclusions were seen after exposure of monolayers to 0.3 μg/ml for 48 h. However, continued incubation in the same concentration of tetracycline for 96 h (schedule b) or withdrawal of the drug after 48 h and further incubation for 48 h (schedule c) both resulted in visible, albeit small, inclusions (Fig. 1C). The appearance of inclusions at 96 h which were not visible at 48 h suggests that inclusion development was slowed rather than prevented at 0.3 μg/ml. This suppressive action of tetracycline on C. trachomatis at concentrations below about 2 μg/ml was confirmed when monolayers were assayed for infectivity (Fig. 1D). Here, tetracycline concentrations of 1.2 to 2.4 μg/ml were needed to prevent reappearance of infectivity on passage if infected monolayers were only exposed to the drug for 48 h (schedules a and c); tetracycline was lethal at a slightly lower concentration if monolayers were exposed for 96 h (schedule b). This inhibitory action of low concentrations of tetracycline on C. trachomatis is illustrated by the discrepancy between the MIC (0.3 μg/ml) and the MLC (2.4 μg/ml).

DISCUSSION

Previous studies have demonstrated that IF staining techniques detect not only mature and immature C. trachomatis inclusions (13, 14), but also the small abnormal inclusions which develop in the presence of β-lactams (unpublished data). In this study, IF proved to be a sensitive stain for visualizing both mature inclusions and the smaller inclusions which developed when chlamydia-infected monolayers were exposed to certain concentrations of ofloxacin and tetracycline. The limit of this technique is imposed by the size of the inclusion rather than the sensitivity of the stain, since below a certain size it is difficult to identify inclusions by fluorescence microscopy at the magnifications used in this work (×100 to ×400).

Our test was designed (i) to determine the MIC of the test drug (schedule a), (ii) to establish whether the MIC was altered if infected monolayers were exposed to the drug for 96 h rather than 48 h (schedule b), and (iii) to predict, without passage experiments, whether a drug at a particular concentration was eradicating or merely suppressing C. trachomatis. To determine this, monolayers were exposed to the drug for 48 h and then reincubated for an additional 48 h after withdrawal of the antimicrobial agent (schedule c). Results of schedules a and c were then compared. Predictions made on the basis of inclusion development in these three schedules were confirmed by assaying monolayers from each schedule for infectivity, and the lowest drug concentration which both inhibited inclusion development and destroyed all infectivity in schedule c was defined as the MLC.

Results obtained with tetracycline demonstrated the potential of this in vitro screening test. It was simpler to perform than either the technique of Ridgway et al. (9), which involves up to 10 “blind” passages after withdrawal of the drug, or the method of Bowie and co-workers (3, 6), in which infected monolayers were exposed to the drug for various times and at different stages of the chlamydial developmental cycle before passage. Nevertheless, our test was at least as informative; the MIC of tetracycline was comparable to that reported previously (5, 6), and the discrepancy between the MIC (0.3 μg/ml) and the MLC (2.4 μg/ml) confirms earlier observations that the action of tetracycline against chlamydia is bacteriostatic rather than bacteircidal (6).

The effect of ofloxacin on C. trachomatis differed dramatically from that of tetracycline, since the MLC was identical to the MIC, and exposure of monolayers to ofloxacin for 96 h produced similar results as exposure for 48 h. Our conclusion is that ofloxacin is lethal when McCoy cells infected with C. trachomatis are exposed to 1 μg/ml throughout one developmental cycle. Similar results have recently been reported with ciprofloxacin, another new quinoline derivative (4).

Serum levels of 1 μg of ofloxacin per ml can be achieved in vivo (10), and our results suggest that studies on the action of this drug against naturally occurring C. trachomatis infections are justified. Moreover, since in vitro tests have shown
*Neisseria gonorrhoeae* to be highly sensitive to ofloxacin (11), it will be interesting to see whether this new antimicrobial agent is effective treatment for patients with mixed infections of *C. trachomatis* and *N. gonorrhoeae*.

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**LITERATURE CITED**


14. Thomas, B. J., R. T. Evans, G. R. Hutchinson, and D. Taylor-Robinson. 1977. Early detection of chlamydial inclusions com-
