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In Vitro Studies of *Chlamydia trachomatis* Susceptibility and Resistance to Rifampin and Rifabutin

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Although rapid emergence of antibiotic-resistant mutants of *Chlamydia trachomatis* occurs when the organism is grown in subinhibitory concentrations of rifampin, no such mutants could be demonstrated when the organism was propagated under the same conditions in subinhibitory levels of the related drug rifabutin.

Rifampin, a potent inhibitor of DNA-dependent RNA polymerase, has been shown to be very active against *Chlamydia trachomatis* in a number of in vitro cell culture studies (2–5, 7). Becker (1) suggested that although a high incidence of rifampin-resistant mutants may occur in tubercular- and meningococcal-carrier states, the appearance of resistant *C. trachomatis* mutants may be less frequent because of the existence of two separate inhibitory sites on the rifampin molecule. However, subsequent studies have shown the rapid emergence of rifampin-resistant chlamydiae when passaged in the presence of subinhibitory levels of the drug (5, 6).

More recently, several new rifamycin derivatives have become available, among which is rifabutin, a chemical congener of rifampin, which is synthesized by the chemical manipulation of rifamycin S. Studies have indicated that rifabutin may have activity against a wider range of organisms than rifampin; that when administered orally, rifabutin reaches much higher levels in tissues than in plasma and thus may be particularly useful for the treatment of intracellular infections; and that emergence of resistance to this new drug may be of much lower frequency than the emergence of resistance to rifampin (9).

Our earlier work showed that there was no substantial difference in the MIC of rifampin when tested against 9 of the 15 *C. trachomatis* serovars (7). We were, however, able to demonstrate a stepwise increase in the MIC of rifampin for *C. trachomatis* which resulted in a 500-fold increase in resistance (MIC, 0.008 to 5.0 μg/ml) during 20 passages of the organism grown in the presence of subinhibitory levels of the drug. The purpose of our present study was to confirm the previous rifampin resistance findings in parallel with similar studies using rifabutin.

A stock of *C. trachomatis* serovar G was grown in mitomycin C-treated McCoy cells (8) and dispensed in 1-ml volumes which were stored at −70°C until required. Each stock sample, when thawed and inoculated into fresh, antibiotic-free McCoy cells, produced 100 to 500 inclusions per 13-mm-diameter cover slip. Mitomycin C-treated McCoy cells had previously been found to produce significantly more chlamydial inclusions than cycloheximide-treated McCoy cells (8). All MICs were determined by methods described previously (7), except that rifampin or rifabutin was added 1 h after centrifugation, the McCoy cells were treated with mitomycin C rather than gamma irradiation, and inclusions were stained with a species-specific fluorescein-labeled monoclonal antibody against *C. trachomatis* (Microtrak; Syva Co. Inc.). All MIC determinations were done in duplicate, and MICs were calculated as the lowest antimicrobial concentration which completely inhibited the formation of either elementary or reticulate body inclusions in cell culture. After determination of the initial MICs of both rifampin and rifabutin, the chlamydiae were passaged in cell cultures which contained no antibiotics except for 0.1 MIC of each of the drugs being tested. After 48 to 60 h of incubation, cultures were harvested, and the chlamydial yields were either stored at −70°C or immediately repassaged into fresh, drug-treated cells. Occasionally, drug-treated cell culture harvests were repassaged in the absence of drug in order to increase the numbers of chlamydial inclusions. This was done especially when drug treatment reduced the inclusion count to less than 100 or when evidence of inhibition of chlamydial replication occurred, as judged by the appearance of atypical inclusions or a very reduced number of elementary bodies within the inclusions. Such passages were excluded from the calculations of the total number of cell culture passages of the organisms under drug treatment. Recalculations of the MICs for drug-treated organisms were undertaken after 5 and 10 passages in the respective drug.

Results showed that the initial MICs of rifampin (0.008 μg/ml) and rifabutin (0.009 μg/ml) were remarkably similar (Table 1). After five passages of *C. trachomatis* in 0.0008 μg of rifampin per ml (0.1 MIC), the retested MIC of rifampin against the drug-treated organisms showed a 20-fold increase (0.16 μg/ml), whereas there was no increase in the MIC of rifabutin after *C. trachomatis* was passaged five times in 0.1 MIC of that drug. After 10 passages of *C. trachomatis* in 0.1 MIC of rifampin, the new MIC of rifampin when retested against the drug-treated organisms was >1.0 μg/ml, representing an increase of over 100-fold. These rifampin-resistant mutants of *C. trachomatis* were stable when repassaged three to four times in the absence of the drug. When the rifabutin-treated chlamydiae were retested against rifabutin after 10 passages, no increase over the original MIC was observed (Table 1). In cross-titration experiments, determination of the MIC of rifabutin against the rifampin-treated chlamydiae after 10 passages also showed an elevated MIC of 0.15 μg/ml (i.e., a 20-fold increase). Conversely, the MIC of rifampin against rifabutin-treated organisms remained at 0.008 μg/ml after both 5 and 10 passages in cell culture.

This work substantiates our original findings and the findings of others that there is a rapid emergence of chlamydial resistance to rifampin. In addition, our data suggest

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TABLE 1. MICs for *C. trachomatis* serovar G

No. of cell culture passages	MIC (μg/ml)	
	Rifampin	Rifabutin
0	0.008	0.009
5	0.16	0.009
10	>1.00	0.009

that there is negligible emergence of *C. trachomatis* mutants resistant to rifabutin or that the rate of emergence is much slower than that of mutants resistant to rifampin under the same in vitro conditions. The results of cross-titrating rifampin-treated chlamydiae against rifabutin may suggest that some partial cross resistance occurs between these two related drugs, although further studies are necessary to clarify the nature of this phenomenon. The longer half-life and lower frequency of emergence of mutants resistant to rifabutin prompt further studies of this drug and a reappraisal of other new rifamycin derivatives in the treatment of chlamydial disease.

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