Activities of First-Choice Antimicrobials against Gamma Interferon-Treated *Chlamydia trachomatis* Differ in Hypoxia

Kensuke Shima,a Matthias Klinger,b Werner Solbach,a Jan Ruppa,c

Institute of Medical Microbiology and Hygiene, University of Lübeck, Lübeck, Germany; Institute of Anatomy, University of Lübeck, Lübeck, Germany; Medical Clinic III/Infectious Diseases, UK-SH/Campus Lübeck, Lübeck, Germany

Gamma interferon (IFN-γ)-mediated host responses play a central role in resolving genital *Chlamydia trachomatis* infections but may also result in persistence of the pathogen, which shows reduced susceptibility to antimicrobials. The antichlamydial function of IFN-γ is oxygen dependent, and the efficacy of antimicrobials against *C. trachomatis* is reduced in a low-oxygen environment. In this study, we show that the antichlamydial efficacies of azithromycin and doxycycline differ in IFN-γ-treated cells under hypoxia.

Urogenital tract infections with *Chlamydia trachomatis* are the most common bacterial sexually transmitted diseases (STDs) in the United States (1). Recurrent infections are associated with chronic inflammatory processes that may result in pelvic inflammatory disease (PID), ectopic pregnancy, and infertility (1, 2). Although azithromycin and doxycycline have been recommended as first-choice antimicrobials for the treatment of genital *C. trachomatis* infection, therapeutic failures have been observed, presumably due to subinhibitory drug concentrations or reduced antimicrobial efficacy at the infection site (3). Experimental data indicate that persistent *C. trachomatis* is less susceptible to doxycycline than productive *C. trachomatis* under normoxia (4). However, it was reported that oxygen concentrations of the cervix and vagina range from 0.5 to 5.5% under physiological conditions and may further decrease during an infectious process (5). In addition, gamma interferon (IFN-γ)-mediated functions against chlamydiae are not as effective at suppressing chlamydial development and progeny in a low-oxygen environment (6). While there is information on antimicrobial effects against IFN-γ-induced persistent *C. trachomatis* under normoxia (4), data on antimicrobial effects against *C. trachomatis* in IFN-γ-treated cells under hypoxia are lacking. We therefore compared the efficacies of azithromycin and doxycycline against *C. trachomatis* in IFN-γ-treated cells under normoxia and hypoxia.

To establish a persistent infection, 0.5 × 10⁵ HeLa cells (ATCC CCL-2) in 24-well plates were treated with IFN-γ (10 U/ml) in RPMI 1640 medium containing 5% fetal bovine serum (FBS) under normoxia (20% O₂) and hypoxia (2% O₂) for 48 h. Then cells were infected with 2.5 × 10⁵ inclusion-forming units (IFUs)/ml *C. trachomatis* L2 (ATCC VR-902B) in the presence of IFN-γ. Twelve hours postinfection (h.p.i.), 0.01 to 0.05 μg/ml azithromycin or 0.03 to 0.1 μg/ml doxycycline was added to the infected cells in the presence of IFN-γ. In preliminary experiments, increasing the antimicrobial concentrations (0.25 μg/ml azithromycin or doxycycline) resulted in more than 99% suppression of *C. trachomatis* growth in the IFN-γ persistence model. After 12 h of incubation, cells were washed and the medium was supplemented with tryptophan (100 μg/ml) to reactivate persistent chlamydiae. *C. trachomatis*-infected cells were harvested after 24 h of reactivation, disrupted with glass beads, and inoculated onto 3 × 10⁵ HEp-2 cells (ATCC CCL-23). Recoverable *C. trachomatis* was visualized by immunofluorescence assay and normalized to the antimicrobial-untreated controls under normoxia and hypoxia. As a control study, the complete procedure was performed in the absence of IFN-γ.

To validate *C. trachomatis* persistent infection, we investigated the inclusion size and the production of progeny under normoxia and hypoxia. Treatment with IFN-γ resulted in persistent infection under normoxia, characterized by smaller inclusions and reduced progeny, whereas large-inclusion formation and infectious progeny were still detected under hypoxia (Fig. 1). As shown pre-

![Graph](http://aac.asm.org) FIG 1 IFN-γ effects on *C. trachomatis* development under normoxia and hypoxia. Under normoxia, IFN-γ treatment resulted in dramatically reduced growth of *C. trachomatis* while growth under hypoxia was hardly suppressed (n = 10; means ± standard errors of the means [SEM] are shown; ***), P < 0.001). Immunofluorescence staining shows chlamydial inclusions (green) and host cells (red).
viously (7), the efficacy of azithromycin and doxycycline was reduced in *C. trachomatis*-infected cells under hypoxia compared to normoxia (Fig. 2B and C). Thus, we further analyzed the antimicrobial efficacy in the IFN-γ-induced persistence model. By electron microscopy (EM), enhanced reticulate body (RB) formation and differentiation to infectious elementary bodies (EBs) were observed in cells treated with azithromycin but not cells treated with doxycycline under hypoxia (Fig. 2A). In accordance with the

![FIG 2](http://aac.asm.org/)

**FIG 2** Efficacy of antimicrobials against *C. trachomatis* in the presence or absence of IFN-γ under normoxia and hypoxia. (A) Representation of the experimental setting in the presence of IFN-γ. Immunofluorescence staining and EM pictures show *C. trachomatis* before and after reactivation under different conditions. Arrowheads show start and endpoints of doxycycline (DOX) or azithromycin (AZM) treatment. Arrows show chlamydial inclusions. White and black bars, 0.6 and 3 μm, respectively. (B to E) Recoverable *C. trachomatis* under AZM (B) (*n* = 5, mean ± SEM) and DOX (C) (*n* = 7; mean ± SEM) treatments in the absence of IFN-γ shows reduced efficacy of both substances under hypoxia compared to normoxia. In contrast, when cells are treated with IFN-γ, the efficacy of AZM (D) (*n* = 10; mean ± SEM) against *C. trachomatis* is decreased under hypoxia compared to normoxia, whereas equal or even better eradication efficacy is observed for DOX (E) (*n* = 12; mean ± SEM) under hypoxic conditions. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.
morphological results, the eradication efficacy of azithromycin (0.03 to 0.05 μg/ml) was significantly reduced under hypoxia (Fig. 2D). On the other hand, doxycycline (0.1 μg/ml) showed the same efficacy in the eradication of C. trachomatis in IFN-γ-treated cells under normoxia and hypoxia and showed higher eradication efficacy under hypoxia when lower concentrations (0.03 and 0.05 μg/ml) were applied (Fig. 2E).

Reduced antimicrobial activity against C. trachomatis has been linked to the persistent state but also to the emergence of bacterial resistance that is mediated by different types of mutations (8). In this study, we compared the efficacies of azithromycin and doxycycline against C. trachomatis in IFN-γ-treated cells under different environmental oxygen concentrations. In the experimental setting, persistent C. trachomatis can be induced by several different factors such as antimicrobial treatment, nutrient deprivation, and host immune responses (9). One of the best-described and most reliable models is IFN-γ-induced persistence. In the T-cell-dependent adaptive immune response against C. trachomatis, IFN-γ-dependent mechanisms predominate (10, 11). In addition, host-pathogen-related transcriptional changes have been well characterized in this model (12). We could show previously that host interactions, Constanza et al. could show that uptake and accumulation of 3H-labeled azithromycin were more efficient than uptake and accumulation of doxycycline under the normoxic condition (4). Our findings further indicate that the efficacy of subinhibitory antimicrobial concentrations against IFN-γ-treated C. trachomatis strongly depends on the oxygen concentrations. Because of the metabolic switch to a glycolytic metabolism, resulting in an increased consumption of glucose and extensive production of lactate and protons, hypoxia is often associated with an acidic state (13). In addition, IFN-γ treatment itself may contribute to increased cellular glucose consumption and lactate production under normoxia, which are further augmented by hypoxia (14). It should therefore be considered that an altered pH may directly affect different mechanisms (e.g., absorption, distribution, excretion) of antimicrobial function in a low-oxygen environment. Furthermore, enhanced stabilization of hypoxia-inducible factor 1 (HIF-1) under hypoxia modulates numerous pathways involved in the regulation of cellular pH and energy metabolism (15). We speculate that the interplay of hypoxia-induced host factors with chemical properties of antimicrobials and the unique chlamydial characteristics in persistence might be the cause for the altered efficacy of antimicrobials under hypoxia. Taking into account the observation that different oxygen concentrations alter antimicrobial efficacy against C. trachomatis in an IFN-γ-induced persistence model, environmental circumstances should be in general more carefully considered to predict treatment outcomes during the course of the disease.

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REFERENCES