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Note

Evaluation of the intracellular activity of drugs against *Mycobacterium abscessus* using a THP-1 macrophage model

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ABSTRACT

We evaluated the antimicrobial effectiveness against *M. abscessus* in a THP-1 cell line model. No intracellular activity was observed when using amikacin or imipenem. A bacteriostatic effect was observed for cefoxitin, clarithromycin and azithromycin. Tigecycline showed the best antibacterial effect by decreasing the intracellular growth up to bactericidal level.

Mycobacterium abscessus is one of the most drug-resistant species of the pathogenic rapidly growing mycobacteria (RGM) group. *M. abscessus* accounts for most cases of pulmonary disease in patients with underlying disorders such as bronchiectasis, cystic fibrosis, gastroesophageal disorders or granulomatous diseases such as sarcoidosis or tuberculosis (Cullen et al., 2000; Griffith et al., 1993; Griffith et al., 2007). *M. abscessus* is also involved in sporadic cases of skin and soft tissue infections (Griffith et al., 2007; Gutiérrez-de la Peña et al., 2010; Maurer et al., 2014); and it is the third most frequently recovered non-tuberculous mycobacteria (NTM) respiratory pathogen in the United States, accounting for approximately 80% of RGM respiratory disease isolates (Griffith et al., 1993). *M. abscessus* may be found in water, soil, surgical instruments and injectable solutions.

The therapy for *M. abscessus* infections is complicated, mainly because of intrinsic and acquired resistance to commonly used antibiotics. Acquired mutational resistance to clarithromycin (23S rRNA gene) and amikacin (16S rRNA gene) can occur easily because *M. abscessus* has a single copy of the rRNA operon (Griffith et al., 2007). Low or intermediate minimal inhibitory concentrations (MICs) are obtained with clarithromycin (100%), tigecycline (100%), amikacin (90%), (Griffith et al., 2007; Wallace et al., 2002) and cefoxitin (70%) for untreated *M. abscessus* isolates. Some isolates (50%) also show low MIC values to imipenem (Griffith et al., 2007). Most of the therapeutic regimens to control symptoms and progression against *M. abscessus* involve high doses and a combination of drugs for long periods, ranging from 2 weeks to 4 months in cases of serious skin or soft tissue infections and reaching up to 6 months for bone infections. For lung infections, longer regimens are required, producing only clinical and microbiological improvement with high associated costs and morbidity (Griffith et al., 2007).

M. abscessus has a limited *in vitro* drug susceptibility spectrum, with little evidence for its correlation between *in vitro* susceptibility data and clinical response to pulmonary disease (Griffith et al., 2007). A possible explanation is that *M. abscessus*, as well as other mycobacteria, is intracellular, and only a few antimicrobials can penetrate the cell-membrane of phagocytes. Therefore, the development of laboratory tests to assay the efficacy of intracellular antibacterial agents may be useful to choose the most effective antimicrobials to prescribe and to test new antimicrobials against this pathogen. The aim of this study was to evaluate an *ex vivo* model of *M. abscessus* infection on human macrophages derived from a THP-1 monocyte cell line to determine the effectiveness of certain commercially available drugs at killing intracellular bacteria.

M. abscessus virulent strain L948 ATCC 19977 (Ordway et al., 2008) was grown on Middlebrook 7H9 broth for 12 h at 37 °C, aliquoted in cryovials and stored at −70 °C until use. Bacterial stocks CFU's were determined by agar plating. Tigecycline (Sigma), clarithromycin (Abbott Laboratories, Ltd.), cefoxitin (USP, Apotex Corp), imipenem (Sigma), azithromycin (Sigma), and amikacin (Sigma) stocks were prepared at 1 mg/ml concentration. The MIC value for each drug for *M. abscessus* L 948 was determined as recommended by the Clinical and Laboratory Standards Institute (CLSI), document M24-A2, using a broth microdilution method (Woods et al., 2011) The concentration drug range was 0.25 to 64 μg/ml. MIC values were determined after 3–5 days

Abbreviations: RGM, rapidly growing mycobacteria; NTM, non-tuberculous mycobacteria; MIC, minimal inhibitory concentrations; MOI, multiplicities of infection; CFU, colony forming unit

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of incubation at 30 °C. Quality control testing was performed using *Staphylococcus aureus* ATCC 29213. Human monocyte cell line THP-1 was cultured and transformed into macrophages as previously described (Castro-Garza et al., 2006). Several multiplicities of infection (MOI's) were tested in order to define the optimal bacterial inoculum to reach intracellular logarithmic growth in the cell cultures after 72 h of incubation. Intracellular anti-bacterial assays were performed as follows: bacteria were thawed at 37 °C and adjusted to 4×10^5 bacteria/ml with RPMI-1640 medium; the monolayers were infected at an MOI of 1:1 (cell:bacillum) and incubated for 6 h at 37 °C in a 5% CO_2 humid atmosphere. In order to remove extracellular bacteria, cultures were washed twice with pre-warmed PBS at 37 °C; then, 1 ml of amikacin (200 μg/ml) was added per well and incubated under the same conditions for 2 h. The wells were washed twice with pre-warmed PBS; then, 1 ml of RPMI-1640 containing the drug was added at concentrations of 0.25×, 1×, 4×, and 16× the MIC value. Three wells were used to quantify the initial colony forming units (CFUs) by agar plating, and the rest of the wells were incubated for up to 72 h. Bacteria were quantified by lysing the macrophages with saline solution-Triton X-100 1%, and the CFUs determined by plating on blood agar after 0, 24, 48, and 72 h of incubation with drugs. Experiments were performed in triplicate, and the data are expressed as the $\log_{10}$. Data were compared by using Bonferroni's multiple comparison post-test ($P < 0.05$).

The MIC values for tigecycline, clarithromycin, azithromycin, imipenem, amikacin, and cefoxitin for the *M. abscessus* L948 ATCC 19977 strain were 1, 1, 8, 16, 16, and 32 μg/ml, respectively. Amikacin and imipenem did not have any intracellular effect on *M. abscessus* infecting THP-1 macrophages (Fig. 1). As shown in Fig. 1, the intracellular CFU count remained constant from time 0 until 72 h for any drug concentration. Cefoxitin and clarithromycin displayed a low antibacterial effect (Fig. 1). Cefoxitin showed a log reduction of 0.5 at 16× MIC; in contrast, clarithromycin revealed a reduction of 1 log at the same concentration. Azithromycin showed a good antibacterial effect, with a reduction of 2 log at the initial CFU (Fig. 1). The best results were obtained with tigecycline (16× MIC), which reduced the intracellular growth to 2.9 log at 72 h ($P < 0.05$), as seen in Fig. 1.

M. abscessus is a facultative intracellular bacterium, and many of the factors and mechanisms involved during infection have been described in an infected macrophage model (Howard et al., 2006). On the other hand, most of the antibacterial assays for mycobacteria are carried out in *in vitro* systems, but few have included models to test the intracellular activity of novel compounds. Skinner et al. (1994) analysed the antimycobacterial activity of established and experimental drugs in intracellular *M. avium* in bone marrow-derived murine macrophages. They were able to distinguish between compounds with bactericidal activity and those with bacteriostatic effects, even if both drugs had similar MICs. Recently, our research group evaluated the activity of two new compounds (tedizolid and ACH-702) against intracellular *M. tuberculosis* in human macrophages. Those drugs had a good intracellular killing activity, comparable to rifampin and moxifloxacin (Molina-Torres et al., 2014).

Byrd and Lyons (1999) first described the model of infection using human monocytes, where a virulent variant of *M. abscessus* was able to persist and multiply inside the monocytes. A susceptibility study using macrophages was done by Greendyke and Byrd (2008); they determined the susceptibility of *M. abscessus* variants in biofilms and human macrophages to clarithromycin, amikacin and cefoxitin, observing minimal or null activity of those compounds against the stationary

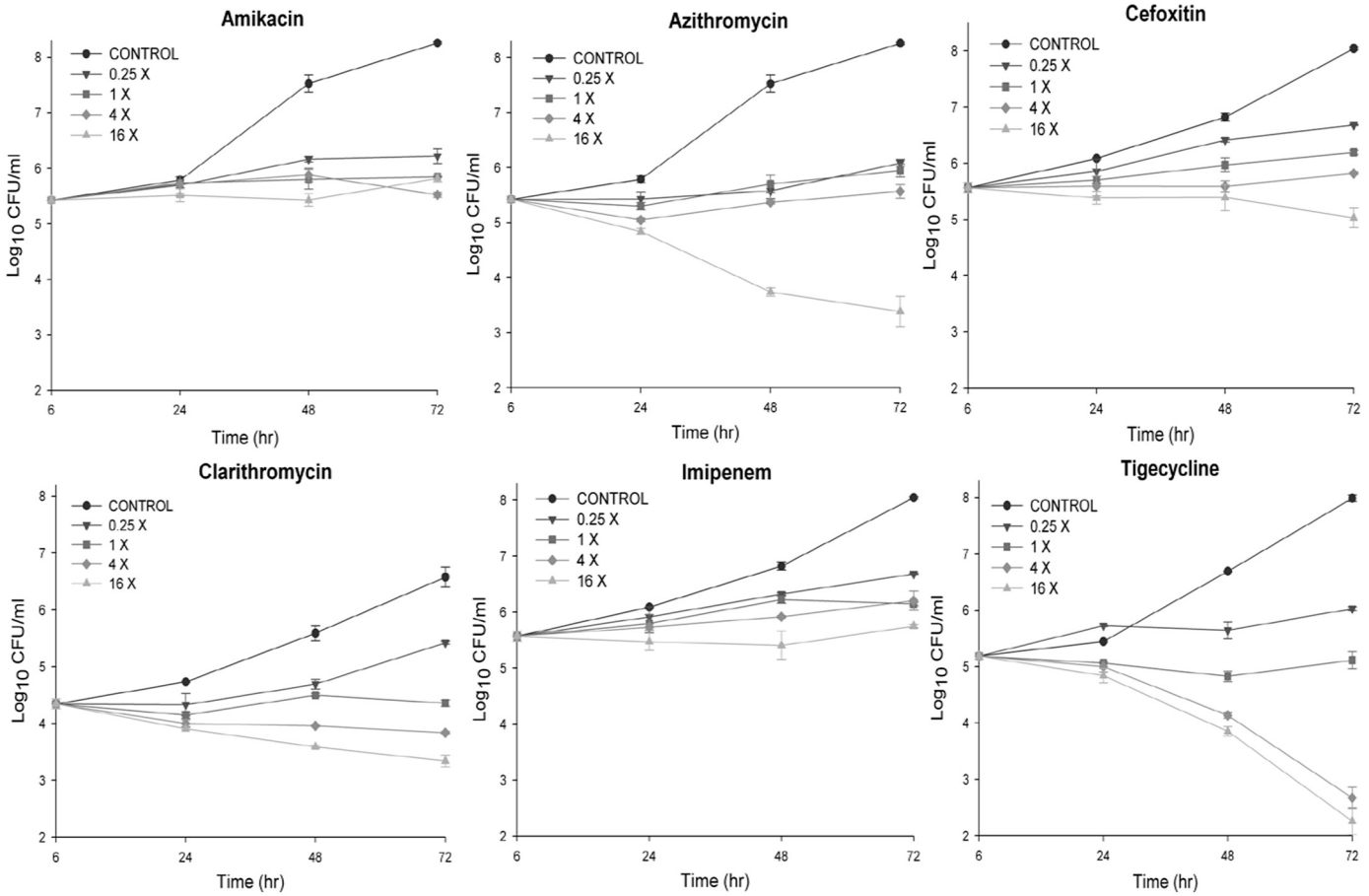

Fig. 1. Intracellular activity of diverse antimicrobials against *M. abscessus* infected THP-1 macrophages monolayer. Bacterial counts were done at 24, 48 and 72 h after infection. Error Bars represents the mean of three measurements.