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Letter to the Editor

Extracellular and intracellular activity of iclaprim against *Listeria monocytogenes*

Editor: Professor Paul Tulkins

Sir,

Listeria monocytogenes is a Gram-positive, non-spore-forming bacillus causing food-borne, life-threatening infections including sepsis and meningoencephalitis in neonates, pregnant women and immunocompromised patients [1]. Approximately one-third of patients with listeriosis die as a result of their infection. Therapeutic failure is probably not due to antimicrobial resistance of this pathogen but rather to the ability of *L. monocytogenes* to actively penetrate into a wide range of host cells [1]. These intracellular bacteria may be concealed from the extracellular environment of high antibiotic concentrations. Diffusion or active transport of an antimicrobial agent into the host cells is therefore an important factor in the treatment of listeriosis. Here, the in vitro extracellular and intracellular activity of iclaprim against *L. monocytogenes* is reported. Iclaprim is a selective bacterial dihydrofolate reductase inhibitor designed to be more active than trimethoprim and to overcome trimethoprim resistance among Gram-positive pathogens [2]. In addition, iclaprim does not need to be combined with a sulfonamide, which is associated with renal toxicity, hepatotoxicity, blood dyscrasia, anaphylaxis and hypersensitivity reactions.

In this study, the extracellular and intracellular activity of iclaprim compared with trimethoprim against *L. monocytogenes* ATCC strain 7302 was conducted using time–kill studies in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [3]. Quality control was performed using *L. monocytogenes* ATCC 7302, and minimum inhibitory concentrations (MICs) were determined using CLSI guidelines [4]. The extracellular activity of iclaprim or trimethoprim (0.25–8 μg/mL) against *L. monocytogenes* was evaluated in time–kill studies in Eagle's Minimum Essential Medium (EMEM; Sigma-Aldrich) supplemented with 10% fetal calf serum, 1% non-essential amino acids and 1% glutamate for 48 h. Gentamicin (100 μg/mL) was used as an internal control. The intracellular activity of iclaprim, measured through cell breakage and CFU counting on agar, was determined in EMEM using 10^5 HeLa cells/mL infected with 5×10^5 CFU/mL *L. monocytogenes* that were subsequently treated with different concentrations of iclaprim or trimethoprim (0.25–8 μg/mL). To determine only the intracellular activity of iclaprim, gentamicin (100 μg/mL), as a rapid bactericidal agent, was added to the medium 3 h post-inoculation to eliminate all non-internalised bacteria. Gentamicin is not active inside the host cell owing to accumulation in lysosomes where it is protonated and sequestered, rendering it antimicrobially inactive [5]. Gentamicin showed no toxicity against eukaryotic cells. Intracellular antimicrobial activities of iclaprim and trimethoprim were determined through CFU counting on agar. No difference in CFU was observed between HeLa cells and those that had been lysed by sonication, dry ice or low osmolarity. HeLa cells were resuspended in Mueller–Hinton broth and were plated directly without additional cell breakage. The number of internalised bacteria in HeLa cells 3 h after inoculation without addition of iclaprim or trimethoprim was estimated to be ca. 10^4 CFU/mL. No cytotoxicity or membrane damage was observed in HeLa cells up to 64 μg/mL iclaprim. HeLa cells remained intact up to 48 h post-inoculation as confirmed by microscopy (trypan blue exclusion test). Iclaprim and trimethoprim MICs against *L. monocytogenes* were 0.06 μg/mL and 0.25 μg/mL. Iclaprim at 0.25–8 μg/mL showed a reduction in CFU of 3 log units after 48 h with extracellular *L. monocytogenes* and a reduction in CFU of 3–6 log units after 48 h with intracellular *L. monocytogenes* (Fig. 1). For comparison, Fig. 1 also shows the extracellular and intracellular activity of trimethoprim.

Iclaprim activity was further studied against 35 non-duplicate *L. monocytogenes* clinical isolates from around the world. The clinical isolates were identified and confirmed using standard bacteriological algorithms and methodologies. All *L. monocytogenes* isolates had an MIC of ≤0.03 μg/mL ($MIC_{50/90}$, ≤0.016/0.03 μg/mL). Iclaprim was ≥32-fold more potent than the standard-of-care penicillin ($MIC_{50/90}$, 0.5/0.5 μg/mL) against this pathogen. The trimethoprim, vancomycin and levofloxacin $MIC_{50/90}$ values were 0.03/0.06, 1/1 and 1/1 μg/mL. The $MIC_{50/90}$ values and MIC range for iclaprim and comparators against the 35 non-duplicate *L. monocytogenes* clinical isolates are available in Supplementary Table S1.

In conclusion, iclaprim demonstrated potent and consistent extracellular and intracellular activity against *L. monocytogenes*. The potent intracellular activity of iclaprim suggests that it penetrates cells well and may accumulate in the cell. Iclaprim may be a potentially useful option in the treatment of listeriosis and merits further investigations.

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Competing interests

DH is an employee of Motif BioSciences Inc.

Ethical approval

Not required.

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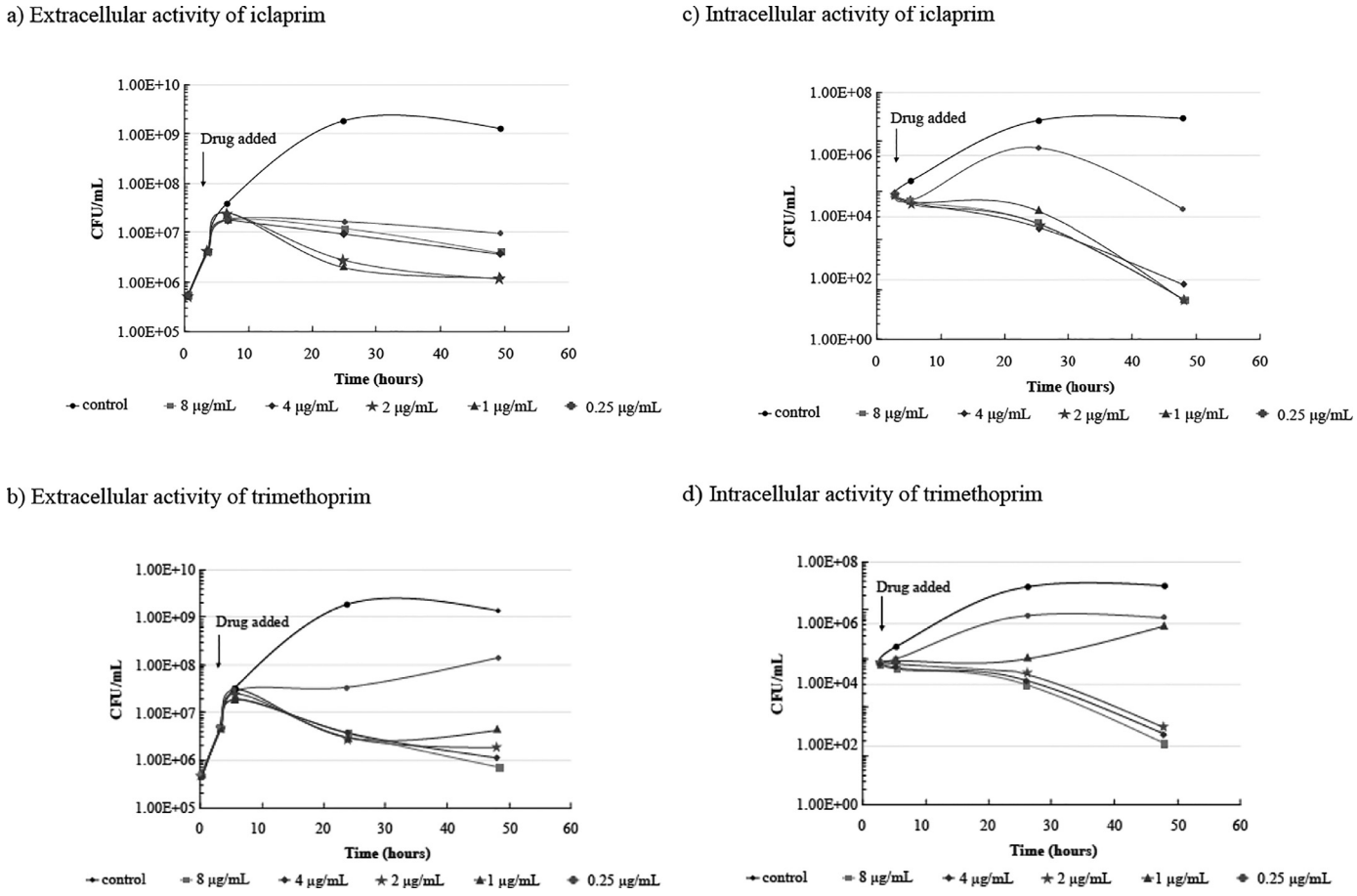

Fig. 1. (a,b) Extracellular and (c,d) intracellular activity of iclaprim (a,c) and trimethoprim (b,d) against *Listeria monocytogenes*.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.09.016.

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