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Short Communication

Mefloquine and its oxazolidine derivative compound are active against drug-resistant *Mycobacterium tuberculosis* strains and in a murine model of tuberculosis infection

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ABSTRACT

Repurposing of drugs to treat tuberculosis (TB) has been considered an alternative to overcome the global TB epidemic, especially to combat drug-resistant forms of the disease. Mefloquine has been reported as a potent drug to kill drug-resistant strains of *Mycobacterium tuberculosis*. In addition, mefloquine-derived molecules have been synthesised and their effectiveness against mycobacteria has been assessed. In this work, we demonstrate for the first time the activities of mefloquine and its oxazolidine derivative compound 1E in a murine model of TB infection following administration of both drugs by the oral route. The effects of associations between mefloquine or 1E with the clinically used antituberculosis drugs isoniazid, rifampicin, ethambutol, moxifloxacin and streptomycin were also investigated. Importantly, combination of mefloquine with isoniazid and of 1E with streptomycin showed a two-fold decrease in their minimum inhibitory concentrations (MICs). Moreover, no tested combinations demonstrated antagonist interactions. Here we describe novel evidence on the activity of mefloquine and 1E against a series of quinolone-resistant *M. tuberculosis* strains. These data show MICs against quinolone-resistant strains (0.5–8 μg/mL) similar to or lower than those previously reported for multidrug-resistant strains. Taking these results together, we can suggest the use of mefloquine or 1E in combination with clinically available drugs, especially in the case of resistant forms of TB.

1. Introduction

Repurposing of drugs to treat tuberculosis (TB) has been considered an interesting alternative to overcome the global TB epidemic, especially to kill drug-resistant forms of the disease [1,2]. Mefloquine (Fig. 1A) is currently used to treat and prevent cases of malaria, and its activity has been largely investigated against other pathogenic organisms such as *Leishmania amazonensis* [3] and *Echinococcus multilocularis* [4].

Of importance, it was previously demonstrated that mefloquine is effective against *Mycobacterium avium* infection [5], also displaying activity in a macrophage model of *Mycobacterium tuberculosis* infection [6]. More recently, mefloquine was suggested as a potent drug to treat multidrug-resistant (MDR) clinical isolates of *M. tuberculosis* [7]. Mefloquine analogue molecules have been synthesised and assessment of their effectiveness against mycobacteria appears to be a promising subject for investigation [8,9].

In this context, a series of mefloquine derivatives was designed, synthesised and evaluated in vitro against *M. tuberculosis*,

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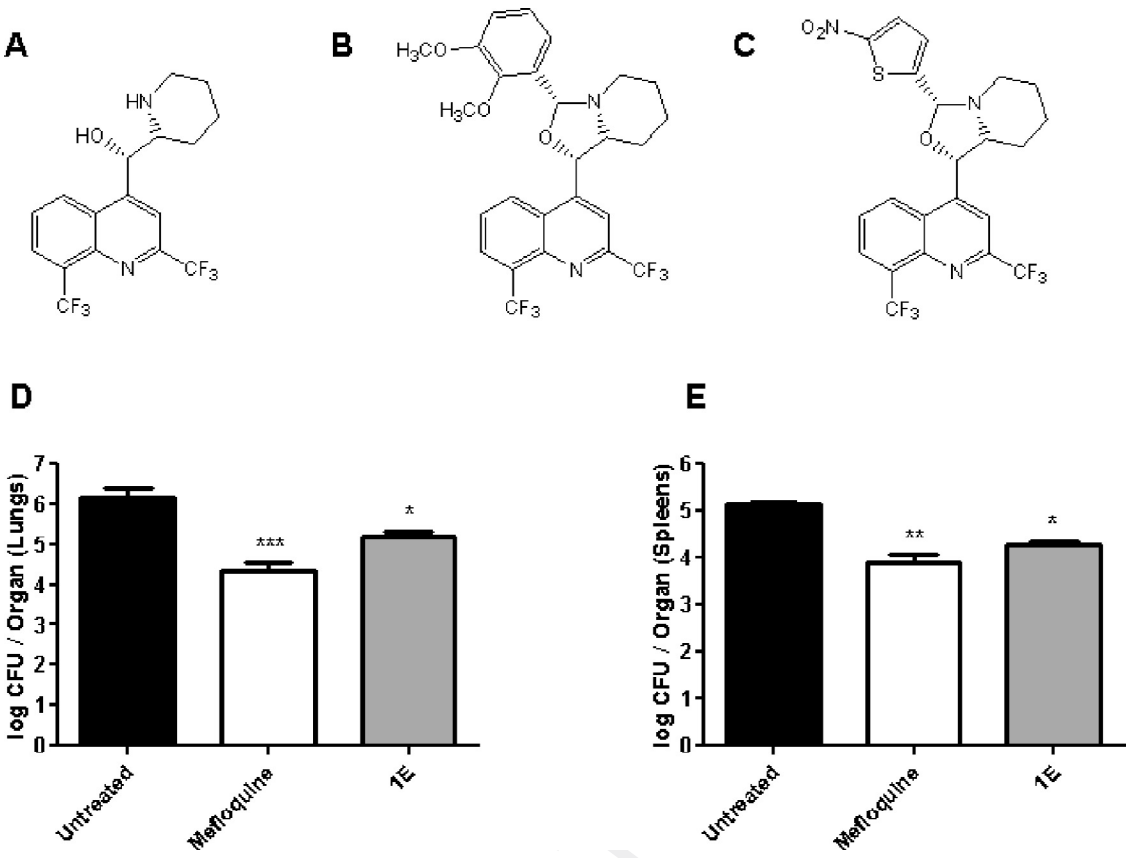

Fig. 1. (A–C) Chemical structures of mefloquine (A) and its derivatives 1E (B) and 2A (C). (D,E) In vivo activity of mefloquine and 1E in a murine model of tuberculosis infection. CFU counts in organ homogenates of the lungs (D) and spleen (E) from mice infected with *Mycobacterium tuberculosis* H37Rv and treated with mefloquine or 1E. Results represent the mean ± standard error of the mean of four mice per group. * $P < 0.05$; *** $P < 0.001$.

showing favourable anti-TB activities and minimal toxic effects to mammalian cells [9]. From this series, compounds 4-[(1S,8aR)-3-(2,3-dimethoxyphenyl)hexahydro[1,3]oxazolo-[3,4-a]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1E; Fig. 1B) and 4-[(1S,8aR)-3-(5-nitro-2-thienyl)hexahydro[1,3]oxazolo-[3,4-a]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (2A; Fig. 1C) were identified as the most promising molecules [9]. They were 2.7 times more active than mefloquine and were less cytotoxic in a cell viability assay in murine macrophages. Furthermore, similar minimum inhibitory concentrations (MICs) were observed compared with ethambutol, a first-line tuberculostatic agent. Therefore, considering these results, compounds 1E and 2A were chosen for further development.

2. Material and methods

2.1. Intracellular activities of mefloquine derivatives in vitro

The intracellular anti-TB activities of 1E and 2A were first assessed. Compounds 1E and 2A were synthesised and characterised according to Gonçalves et al. [9]. The RAW 264.7 macrophage cell line was cultured in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% heat inactivated foetal bovine serum (FBS) (Gibco) and 1% penicillin–streptomycin (Gibco) at 37 °C with 5% CO_2. For the assessment of in vitro activity, macrophages were seeded in 24-well culture plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland) at a density of 3×10^4 cells/well in RPMI 1640 medium (10% FBS without penicillin–streptomycin) and were incubated for 24 h at 37 °C with 5% CO_2. Infection of RAW 264.7 cells with *M. tuberculosis* H37Rv was performed at a multiplicity of infection (MOI) of 1:1 (bacteria/macrophage) for 3 h at 37 °C with 5% CO_2 [10]. The inoculum of *M. tuberculosis* was estimated after CFU determination in solid medium as previously described [11]. Infected RAW 264.7 cells were washed with sterile 0.9% saline to remove extracellular bacteria and the medium was replaced with 1 mL of fresh RPMI (10% FBS without penicillin–streptomycin) [10]. Cells were then treated with 1E or 2A (both at 10 μg/mL) in RPMI medium containing a final concentration of 1% dimethyl sulphoxide (DMSO) (Sigma, St Louis, MO). Untreated control wells received 1% DMSO. After 5 days of incubation, each well was gently washed with sterile 0.9% saline and the infected macrophages were then lysed with 0.025% sodium dodecyl sulphate (SDS) (Invitrogen, Auckland, New Zealand) [10] dissolved in sterile 0.9% NaCl solution. Lysates were serially diluted and were plated on Middlebrook 7H10 agar (Difco, Sparks, MD) supplemented with 10% OADC (oleic acid–albumin–dextrose–catalase) enrichment (Becton Dickinson, Sparks, MD). Bacterial colonies were counted following incubation of the plates for 3 weeks at 37 °C. The experiment was performed in triplicate and the results are expressed as the log mean number (±standard deviation) of bacteria per well of two independent experiments. Data were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni's post-test using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA).

2.2. In vivo analysis of mefloquine and 1E in a murine model of tuberculosis infection

Following the in vitro protocols, the in vivo activity of 1E was analysed using a murine model of TB infection. Twelve 2-month-

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