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Activity of drugs against dormant *Mycobacterium tuberculosis*

A. Iacobino, G. Piccaro, F. Giannoni, A. Mustazzolu, L. Fattorini *

Istituto Superiore di Sanità, Department of Infectious, Parasitic and Immune-Mediated Diseases, Rome, Italy

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ABSTRACT

Objective/background: Heterogeneous mixtures of cellular and caseous granulomas coexist in the lungs of tuberculosis (TB) patients, with *Mycobacterium tuberculosis* (*Mtb*) existing from actively replicating (AR) to dormant, nonreplicating (NR) stages. Within cellular granulomas, the pH is estimated to be less than 6, whereas in the necrotic centres of hypoxic, cholesterol/triacylglycerol-rich, caseous granulomas, the pH varies between 7.2 and 7.4. To combat TB, we should kill both AR and NR stages of *Mtb*. Dormant *Mtb* remodels lipids of its cell wall, and so lipophilic drugs may be active against NR *Mtb* living in caseous, lipid-rich, granulomas. Lipophilicity is expressed as log*P*, that is, the logarithm of the partition coefficient (*P*) ratio $P_{octanol}/P_{water}$. In this study, the activity of lipophilic drugs ($\log P > 0$) and hydrophilic drugs ($\log P \leqslant 0$) against AR and NR Mtb was measured in hypoxic conditions under acidic and slightly alkaline pHs.

Methods: The activity of drugs was determined against AR *Mtb* (5-day-old aerobic cells: A5) and NR *Mtb* (12- and 19-day-old hypoxic cells: H12 and H19) in a Wayne dormancy model of *Mtb* H37Rv at pH 5.8, to mimic the environment of cellular granulomas. Furthermore, AR and NR bacilli were grown for 40 days in Wayne models at pH 6.6, 7.0, 7.4, and 7.6, to set up conditions mimicking the caseous granulomas (hypoxia + slightly alkaline pH), to measure drug activity against NR cells. *Mtb* viability was determined by colony-forming unit (CFU) counts.

Results: At pH 5.8, lipophilic drugs (rifampin, rifapentine, bedaquiline, PA-824, clofazimine, nitazoxanide: $\log P \geqslant 2.14$) reduced CFU of all cells (H12, H19, and A5) by $\geqslant 2\log_{10}$. Among hydrophilic drugs (isoniazid, pyrazinamide, ethambutol, amikacin, moxifloxacin, metronidazole: $\log P \leqslant 0.01$), none reduced H12 and H19 CFUs by $\geqslant 2\log_{10}$, with the exception of metronidazole. When *Mtb* was grown at different pHs the following *Mtb* growth was noted: at pH 6.6, AR cells grew fluently while NR cells grew less, with a CFU increase up to Day 15, followed by a drop to Day 40. AR and NR *Mtb* grown at pH 7.0, 7.4, and 7.6 showed up to 1 $\log_{10}$ CFU lower than their growth at pH 6.6. The pHs of all AR cultures tended to reach pH 7.2–7.4 on Day 40. The pHs of all NR cultures remained stable at their initial values (6.6, 7.0, 7.4, and 7.6) up to Day 40. The activity of drugs against H12 and H19 cells was tested in hypoxic conditions at a slightly alkaline pH. Under these conditions, some lipophilic drugs were more active (>5 log CFU decrease after 21 days of exposure) against H12 and H19 cells than clofazimine, nitazoxanide, isoniazid, pyrazinamide, amikacin (<1 log CFU decrease after 21 days of exposure). Testing of other drugs is in progress.

* Corresponding author at: Istituto Superiore di Sanità, via Regina Elena 299, 00161 Rome, Italy.
E-mail address: lanfranco.fattorini@iss.it (L. Fattorini).

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Conclusion: Lipophilic drugs were more active than hydrophilic agents against dormant *Mtb* in hypoxic conditions at pH 5.8. The Wayne model under slightly alkaline conditions was set up, and in hypoxic conditions at a slightly alkaline pH some lipophilic drugs were more active than other drugs against NR *Mtb*. Overall, these models can be useful for testing drug activity against dormant *Mtb* under conditions mimicking the environments of cellular and caseous granulomas.

Conflicts of interest

The authors have no conflicts of interest to declare.

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