Contribution of efflux activity to isoniazid resistance in *Mycobacterium tuberculosis* complex

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The emergence of multi and extensively drug resistant tuberculosis (MDRTB and XDRTB) has increased the concern of public health authorities around the world. MDRTB is defined by the World Health Organization as tuberculosis (TB) caused by organisms resistant to at least isoniazid (INH) and rifampicin (RIF), the main drugs used in TB therapy. INH resistance is mainly due to mutations in the *katG* and *inhA* genes. However, approximately 20-30% of INH resistant *Mycobacterium tuberculosis* isolates do not have mutations in any of the genes associated with INH resistance. This suggests that other mechanism(s) are involved in the development of INH resistance, namely efflux pump (EP) systems, able to extrude the drug to the exterior of the cell, preventing access to its target. We have previously induced clinical INH susceptible *M. tuberculosis* isolates and the H37Rv (ATCC27294) reference strain to high-level resistance to INH, by gradual exposure to increasing concentrations of this drug. In the present study, we have characterized these strains with respect to their EP activity and its contribution to INH resistance.

M. tuberculosis strains and *Mycobacterium bovis* BCG induced to INH resistance were evaluated for their susceptibility to INH in the presence and absence of the EP inhibitors (EPIs) thioridazine, chlorpromazine, verapamil and reserpine, using the BACTEC MGIT 960 system, equipped with the Epicenter V5.53A software and the TB eXIST module. The EP activity was assessed by a real-time fluorometric method that uses ethidium bromide (EtBr), a known EP substrate. The expression level of *M. tuberculosis* EPs genes was quantified by real-time qRT-PCR.

The EPIs decreased INH resistance in the induced strains. In particular, verapamil promoted reversal of resistance to susceptibility levels for some of the strains tested. The same EPIs were able to reduce real-time EtBr efflux. Finally, compared to the non-induced controls, the INH-induced strains showed overexpression of EPs genes. Altogether, these results correlate efflux activity with INH resistance in *M. tuberculosis*.

This study demonstrates that EPs play an important role in INH resistance and, ultimately, on the emergence of MDRTB. Compounds that inhibit EP activity may prevent the development of this resistance and provide the basis for new anti-mycobacterial compounds.