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SMARt (Small Molecule Aborting Resistance) in combination with ethionamide resets innate and acquired ethionamide resistance in Mycobacterium tuberculosis

Alain Baulard[1], Benoit Deprez[2], Marc Gitzinger[3], Christian Kemmer[3], Sergio Lociuro*[3], Vincent Trebosc[3], Nicolas Willand[2]

[1]*Univ. Lille, Cnrs, Inserm, Chu Lille, Institut Pasteur de Lille, U1019-Umr8204-Ciil; Center for Infection and Immunity of Lille*

[2]*Univ. Lille, Inserm, Institut Pasteur de Lille, U1177; Drugs and Molecules for Living Systems*

[3]*Bioversys AG*

Background:

Ethionamide (ETH) is an anti-tubercular drug used as second-line therapy due to the high doses needed for efficacy, which narrow the therapeutic window. Pro-drug ethionamide requires intracellular activation by EthA, a bacterial mono-oxygenase. Transcriptional repressor EthR controls the expression of EthA and, consequently, limits the conversion and activity of ETH. Inhibitors of EthR showed strong effect in boosting EthA production and ETH sensitivity. Using a combination of phenotypic and molecular assays, we identified compounds increasing ETH sensitivity independently of EthA. Here we report the discovery of EthR2/EthA2 as an alternative bio-activation pathway of ETH

Material/methods:

Transcriptomic analysis was carried out on *M. bovis* BCG Pasteur. MIC determinations against panels of ETH-resistant, MDR and XDR *Mycobacterium tuberculosis* (Mtb) clinical strains was performed with Bactec™ Mgit™960

In vivo proof of concept was achieved with swiss (SPF-OF1) female mouse infected by aerosol with 10^5 H37Rv or with ETH-resistant *M. tuberculosis* bacilli.

Results:

In previous reports, we developed series of compounds that specifically inhibit transcriptional repressor EthR to enhanced production of the monooxygenase EthA. During chemical diversification and optimization of these series, we identified compounds enhancing ETH activity without interfering with the conventional pathway EthR-EthA.

Transcriptomic analysis with these compounds led us to the discovery of a cryptic alternative bio-activation pathway of ETH, named EthR2/EthA2, which somewhat mirrors EthR-EthA pathway.

MIC determinations were carried out on a panel of 20 MDR *Mycobacterium tuberculosis* clinical isolates resistant to ethionamide. MIC range of ethionamide alone was 8-256 µg/ml (ethionamide MIC against H37Rv control strain is 2 µg/ml).

The presence of 10 µM of the EthR inhibitor BDM41906 lowered the MIC of ethionamide against control strain H37Rv down to 0.01 µg/ml and between 0.125 and 4µg/ml against 13 strains; MICs against 7 MDR remained high, between 8 and >64 µg/ml. All the 7 MDR strains were confirmed to be EthA mutated. On the contrary, in the presence of 10 µM of the EthR2 inhibitor, the MIC of ethionamide against all 20 strains dropped down to 0.05-0.5 µg/ml. This Small Molecules Aborting Resistance to ETH was then called SMARt-420.

Efficacy experiments in mice infected with an EthA-mutated ETH-resistant Mtb showed that the the administration of SMARt-420 in combination with ETH induced a 4.5 log reduction in lung bacterial burden, whereas high dosage of ETH given alone was completely inefficient.

Conclusions:

Inhibition of EthR was previously shown effective in potentiating the activity and reverting “innate” resistance to ethionamide in Mtb. The discovery of SMARt-420 revealed a cryptic alternative bio-activation pathway of ethionamide somewhat mirroring EthR/EthA. Development of inhibitors of EthR2 resetting innate and acquired resistance to ethionamide opens perspectives of using ETH as first-line anti-TB treatment.