OHet72 and Current Tuberculosis Treatments: Identifying Synergy

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BACKGROUND

- Tuberculosis (TB) is the second leading cause of death from a single infectious agent, after COVID-19.[1]
- Drug susceptible TB is treated with oral administration of 4 drugs for 6-9 months, whereas multi-drug resistant TB requires 5-10 drugs administered for 18-24 months (figure 1). Both regimens cause severe adverse effects.

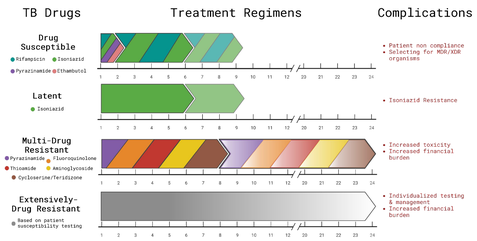


Figure 1: Graphical depiction of current TB treatments[2] indications, regimens, and complications

- OHet72 is a novel anti-cancer compound[3] that also has potent anti-TB activity *in vitro* with no apparent side effects

METHODS

- The Microtiter alamar blue assay (MABA)[4] was used to determine the minimum inhibitory concentration (MIC) of OHet72, first line TB drugs (isoniazid, rifampicin, pyrazinamide), and second line TB drugs (levofloxacin, moxifloxacin).
- Each drug was diluted, and bacteria was added. MABA reagent was added after 1 week. The %reduction of reagent was calculated, and the MIC was considered the lowest concentration of drug that resulted in no bacterial growth.

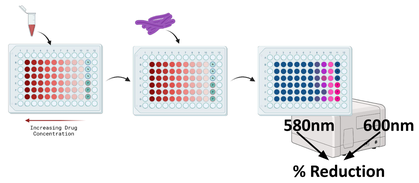
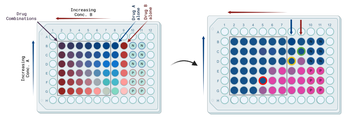


Figure 2: Microtiter alamar blue assay (MABA) method

- Drug interactions were investigated using a MABA checkerboard assay[5]. Two drugs are diluted in different orientations and drug interactions are quantified by calculating the fractional inhibitory concentration index (FICI)[6]



$$FICI = \frac{\text{MIC A in combo}}{\text{MIC A alone}} + \frac{\text{MIC B in combo}}{\text{MIC B alone}}$$

Figure 3: Checkerboard MABA assay template (left), representation of results (right), and fractional inhibitory concentration index (FICI) equation (bottom)

Aim

Identify the type of drug interaction (synergy, addition, or antagonism) that may exist between OHet72 and five drugs currently used against Mycobacterium tuberculosis (MTB).

RESULTS

Table 1: Anti-TB drugs, their experimental MIC determined by the MABA assay against H37Ra MTB, and the reported literature values[7].

Drug	Experimental MIC (µg/mL)	Literature MIC (µg/mL)
Isoniazid	0.1149 ± 0.046	0.1 – 0.2
Rifampicin	0.002 ± 0.00	0.004 – 0.032
Pyrazinamide	240.0 ± 83.56	16 – 512
Levofloxacin	0.3125 ± 0.00	0.125 – 0.5
Moxifloxacin	0.078 ± 0.00	0.032 – 8.0
OHet72	0.929 ± 0.5223	---

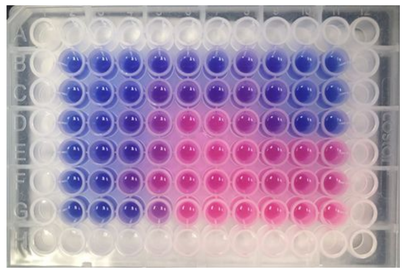


Figure 4: Results of OHet72 and rifampicin checkerboard MABA assay

Table 2: FICI cutoff values for synergistic, additive, and antagonistic drug interaction

FICI Value	Drug Interaction
> 4	Antagonism
1 - 4	Addition
< 1	Synergy

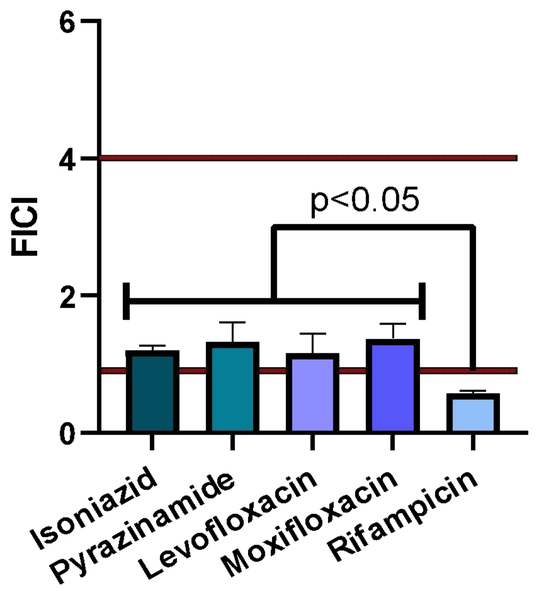


Figure 4: FICI values for each TB drug paired with OHet72 (n=3). Significance determined using one way ANOVA with Tukey's multiple comparisons test

Discussion

- Synergy describes the phenomenon where two or more drugs in combination have a therapeutic effect greater than the sum of the effects of the individual drugs[8]
- The actual drug concentrations of OHet72 and rifampicin in combination that are required to elicit a given therapeutic effect are lower than the expected concentrations based on the effects when the drugs are administered alone

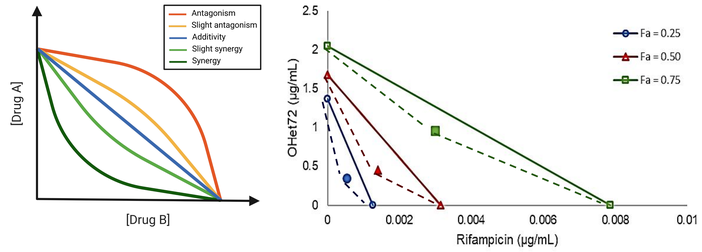


Figure 5: (A) Graphical representation of synergistic (green lines) and antagonistic (orange/red lines) drug pairings compared to and additive reference (blue line); and (B) an isobologram of OHet72 and rifampicin pairing showing drug combinations required to reach specified therapeutic effects in an additive model (solid lines) and the actual concentrations of the drug required to elicit the same effects (dashed lines)

CONCLUSION

- OHet72 would be most effective when administered with rifampicin due to the synergistic drug interactions
- OHet72 has additive interactions with isoniazid, pyrazinamide, levofloxacin, & moxifloxacin
- OHet72 may target MTB cell wall

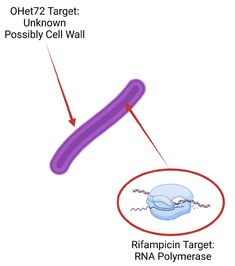


Figure 6: Hypothesis of OHet72 mycobacterial target

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