Mechanism of Action of Clofazimine

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History of research on the mechanism of action of clofazimine

- Barry et al. in 1957: noted that CFZ is a redox active compound with an $E_m$ of -180 mV

- has the potential to be reduced and oxidized within the bacterium, likely in conjunction with electron transport chain (ETC) activity

- oxidation of dyes like CFZ was known to produce peroxide, it was argued that the generation of ROS was a part of the drug's mechanism of action

- supporting this notion, mycobacteria deficient in catalase activity showed a greater sensitivity to CFZ than wild type bacteria

Were the old guys right?

ATP Synthesis: Oxidative Phosphorylation System of *Mycobacterium tuberculosis*

Reduction of Clofazimine by Mycobacterial Type 2 NADH:Quinone Oxidoreductase: A Pathway for the Generation of Bactericidal Levels of Reactive Oxygen Species

Takahiro Yano, Sacha Kassovska-Bratinova, J. Shin Teh, Jeffrey Winkler, Kevin Sullivan, Andre Isaacs, Norman M. Schechter, and Harvey Rubin

CFZ restores NADH oxidase activity in isolated *M. smegmatis* membranes (a) but not in Gram negative membranes (b)

**Specificity**
1. no reduction of CFZ with succinate
2. inhibited by TPZ
3. not seen in Gram negative organisms
4. seen with purified enzyme
Redox cycling of CFZ in reactions with M. smegmatis membranes

Yano T et al. J. Biol. Chem. 2011;286:10276-10287
ROS production catalyzed by M. smegmatis log-phase membranes in the absence and presence of CFZ.: demonstration of CFZ-mediated ROS production.

Yano T et al. J. Biol. Chem. 2011;286:10276-10287
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Figure 10. Inhibition of *M. smegmatis* growth by CFZ and INH and effect of antioxidants. (a) Effect of CFZ on colony counts of wild type *M. smegmatis* mc² 155 plated on 7H9-agar. Data at each concentration is the average colony number on 3 plates ±SD. (b) Effect of free radical scavengers on CFZ inhibition of *M. smegmatis* growth. *M. smegmatis* was grown on agar plates containing various antioxidants (5.0 mM 4-Hydroxy-TEMPO, 5.0 mM NAC and 12.5 μg/mL α-tocopherol) with or without 0.5 μg/mL CFZ or 10 μg/mL INH. Representative plates demonstrating bacterial growth under the various conditions are shown.
Recent insights from Dr. Hung’s lab


“We used the antibiotic clofazimine, which results in increased intracellular ROS production due to redox cycling (38). CFZ, recently identified as a competing substrate for the enzyme NDH-2 within the electron transport chain, is reduced by NDH-2 and subsequently oxidized by O2, resulting in increased ROS (38). When we challenged persisters with CFZ, the number of surviving persisters decreased by three orders of magnitude, contrasting with the tolerance observed for rifampin and streptomycin (Fig. 5B). In the presence of thiourea the addition of CFZ did not change the number of persisters in a statistically significant manner, suggesting that CFZ is killing the persister population through increased intracellular ROS generation.”

**Fig. 5.** Modulating the free-radical concentration affects the number of persisters identified. (A) *M. smegmatis* cells treated with CIP and INH in open, aerated conditions in the presence (dashed line) or absence (solid line) of the free-radical quencher thiourea at a concentration of 150 mM. The presence of thiourea restores a biphasic kill kinetic under open aerobic conditions and a persister population is again identified. (B) The *M. smegmatis* persister population identified after antibiotic treatment with CIP and INH is killed with the addition of CFZ at day 3.
How does all this work?

a, Two ubiquinone molecules bind to Ndi1. The ubiquinone-binding sites in the Ndi1–ubiquinone complex are shown in ribbon representation. The ubiquinone and FAD molecules are shown as sticks and labelled b, Ribbon representation of the reaction centre in the Ndi1–NADH–ubiquinone complex

Clofazimine treatment for Mycobacterial infection

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BUT, when the patient was treated with CFZ...
After 4 months of treatment with Azithromycin + Clofazimine

The Penn ID group now has 8 patients on clofazimine for resistant M. chelonae/abscessus/massiliense.

Pictures provided by Dr. Pablo Tebas