Molecular characterization of pyrazinamide mode of action against Mycobacterium tuberculosis

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains one of the world's leading causes of death due to an infectious agent. Among the first-line drugs available, pyrazinamide (PZA) plays a crucial role in the treatment of TB. PZA is a prodrug, which needs to be converted by the bacterial enzyme PncA into pyrazinoic acid (POA), its active form, to exhibit antibacterial activity. Two main and conflicting modes of action are currently proposed in the literature. In the historical model, PZA/POA molecules are mainly active under acidic environmental pH conditions where they acidify intra-bacterial pH (pH_{IB}). However, recent studies have reported a new pH-independent model in which POA inhibits PanD, an aspartate decarboxylase involved in the Coenzyme A (CoA) biosynthesis pathway. Thus, the molecular mode of action of this antibiotic remains poorly characterized.
The aim of my PhD project is to decipher the contribution of acidic pH and the PanD protein in the molecular mode of action of PZA/POA molecules and their efficacy. To answer these questions, we carried out antimicrobial susceptibility testing in chemically-define media and further monitored the effect of PZA/POA molecules on Mtb pH_{IB} expressing a fluorescent reporter. Our results show that acidic pH potentiates the antimicrobial activity of PZA/POA, and disrupts Mtb pH_{IB} homeostasis in a dose-dependent manner. Finally, we show that exogenous pantothenate supplementation, a critical substrate involved in the CoA biosynthesis pathway downstream of PanD, strongly antagonized the antibacterial activity of PZA/POA regardless of the environmental pH, suggesting that this pathway may play a primary role in PZA/POA susceptibility. Such investigations aim at redefining PZA mode of action, and therefore providing new insights into the fundamentals of anti-TB therapy with important clinical implications.