Development of prodrugs of pyrazinoic acid for treatment of tuberculosis

Luis Constantino

Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Email: constant@ff.ul.pt

Tuberculosis (TB), a infectious disease caused by the bacterial pathogen *Mycobacterium tuberculosis* is a leading infectious cause of morbidity and mortality world-wide. As alternatives to circumvent resistance are urgently needed a viable approach is the development of drugs from molecules already available. This approach seems to be particularly suited for the first line drug pyrazinamide.

This compound is very important in current therapeutic arsenal as it kills a population of semidormant tubercle bacilli residing inside the macrophages that are not killed by other drugs. Pyrazinamide is itself a prodrug that needs to be activated by a mycobacterial pyrazinamidase in order to be converted into the active metabolite pyrazinoic acid. However mutations in the M. tuberculosis pncA gene, abrogated the pyrazinamidase activity and were shown to be responsible for pyrazinamide resistance.

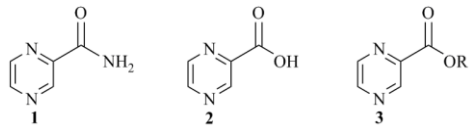

Fig.1 - Pyrazinamide (PZA), Pyrazionoic acid (POA) and Pyrazinoate esters.

Since pyrazinamide is a prodrug, then other prodrugs of pyrazinoic acid activated by different enzymes could indeed be used to deliver the active metabolite inside the mycobacterial cells. Therefore, in this study, we synthesized a series of esters of POA, with different alkoxy chains and evaluate the stability, the activation and the activity of the compounds. By using a prodrug that is activated by esterases, resistance to pyrazinamide could be circumvented as mycobacteria have a great array of esterases that could be used. A central issue regarding the development of ester prodrugs is that these compounds must be unaffected by human esterases during the transport phase but should be readily hydrolyzed by mycobacteria at the site of action.

It was found that all of the esters were active *in vitro* and could also be activated by mycobacterial esterases to release the active drug. Some of the prodrugs were not suitable for *in vivo* use due to poor plasma stability, however, plasma stability could be increased by manipulating the structure of the prodrug and also by incorporating the prodrug in liposomes. Incorporation in these vesicles also increased the activity of the tested prodrug formulation showing that liposomal incorporation is a suitable approach to deliver pyrazinoic acid to macrophages. The most promising compounds were tested in vivo in *M. tuberculosis* and *M. avium* models of infection and showed activity higher than pyrazinamide.

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