Pharmaceutical Regulatory Affairs 2012: Stability, activation and antymycobacterial activity of pyrazinoic acid esters- University of Lisbon, Portugal

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Abstract

Tuberculosis is a leading infectious cause of morbility and mortality world-wide, especially in developing countries Pyrazinamide (PZA), a first line agent for the treatment of tuberculosis, is itself a prodrug that requires activation by the bacterial pyrazinamidase to form its active metabolite pyrazinoic acid (POA) 2, which has poor absorption and significant serum binding. Resistance to PZA is attributed to mutations in the mycobacterial gene encoding pyrazinamidase. Since PZA is in fact a prodrug of POA, other prodrugs, like esters 3, could have activity against M. tuberculosis. Esters of POA have been proposed in the past as alternatives to PZA however the most promising compounds were rapidly degraded in the presence of serum. In order to obtain compounds that could survive during the transport phase, we synthesized lipophilic ester and amide POA derivatives, studied their activity against M. tuberculosis, their stability in plasma and rat liver homogenate and also their activation by a mycobacterial homogenate. The new lipophilic ester prodrugs were found to be active in concentrations 10-fold lower than those needed for PZA to kill sensitive M. tuberculosis and also have a suitable stability in the presence of plasma. Amides of POA although more stable in plasma have lower activity. The reason can probably be found in the rate of activation of both types of prodrugs; while esters are easily activated by mycobacterial esterases, amides are resistant to activation and are not transformed into POA at a suitable rate. These esters could circumvent resistance to PZA because they are activated by different mycobacterial enzymes. In order to be effective in vivo, these compounds must be resistant to hydrolysis by the human enzymes ? (eg. plasma and liver esterases) - but should be readily hydrolyzed by the mycobacterial enzymes at the site of action. Using model esters we were able to select appropriate groups that slow plasma and liver hydrolysis of the prodrugs but do not affect the mycobacterial activation of the compounds and we pursued the work with the synthesis of a series of lipophilic esters. Results on the stability, plasma hydrolysis and antimycobacterial activity of the prodrugs will be presented.

Substituted pyrazinoic acid esters have previously been reported to have in vitro activity against Mycobacterium avium and Mycobacterium kansasii as well as Mycobacterium tuberculosis. Modification of both the pyrazine nucleus and the ester functionality was successful in expanding the antimycobacterial activity associated with pyrazinamide to include M. avium and M. kansasii, organisms usually not susceptible to pyrazinamide.