TITLE: Possible piperine binding site in the *Mycobacterium tuberculosis* RNA polymerase and inhibition of transcription

AUTHORS: [1]MURASE, L. S.; [1]SOUZA, J. V. P.; [1]MENEGUELLO, J. E., [2]SEIXAS, F. A. V.; [1]PALOMO, C. T.; [1]CALEFFI-FERRACIOLI, K. R.; [1]SCODRO, R. B. L.; [1]SIQUEIRA, V. L.; [1]CARDOSO, R. F.

INSTITUTION: [1]Universidade Estadual de Maringá (Avenida Colombo, 5790, CEP 87020-900, Maringá, Paraná, Brasil); [2]Universidade Estadual de Maringá (Avenida Ângelo Moreira da Fonseca, 1800, CEP 87506-370, Umuarama, Paraná, Brasil).

ABSTRACT

Tuberculosis (TB) remains as the infectious disease with the highest mortality and morbidity rates in the world. Treatment for TB is effective, but it is time consuming and can cause significant side effects to the patient. Piperine (PIP) is an alkaloid isolated from *Piper longum* and *Piper nigrum* and is among the prominent candidates as adjunct drug for anti-TB treatment. Besides being a bioavailability enhancer, PIP has shown antimicrobial properties and has been proven as an efflux pumps (EPs) inhibitor in mycobacteria. The aim of this study was to evaluate the potential PIP binding site in the *Mycobacterium tuberculosis* (*Mtb*) RNA polymerase (RNAP) active site. The crystal structure of *Mtb* transcription initiation complex, in complex with the ligands rifampicin (RIF) and N-alpha-(benzenecarbonyl)-N-(2-methylphenyl)-D-phenylalaninamide (88G) were used in the docking simulations with Autodock 4.2.3 and Molegro-6.0 software. Docking programs and protocols were validated by redocking the structure of 88G from pubchem (CID 2206555). The 3D structure of the ligands PIP (zinc 1529772), RIF (zinc 94313219) and 88G (CID 126476624) were downloaded in *.sdf form. All simulations were carried out five times and the results were reproducible in all of them. Structural analysis revealed that PIP shares Tanimoto indexes of 0.173 with 88G and 0.152 with 88D, while 88G and 88D shares 0.779 with each other. Piperine and ligands 88D and 88G showed comparable docking scores in both programs tested, in five independent analyses. In the mechanism proposed in this work, PIP fits in a pattern consistent with crystallographic ligand 88G, with a relatively high score that reinforces our hypothesis. Since PIP and RIF have different binding sites on RNAP, there is no possibility of cross-resistance, and PIP remains active in RIF-resistant isolates, regardless of any mutation present in the RIF binding site on RNAP. We suggest that PIP could interfere in the *Mtb* growth through inhibition of transcriptional activity, differently from what was previously credited only to EPs inhibition. The aforementioned characteristics are very important to consider PIP to compose a new anti-TB strategy.

Keywords: tuberculosis, piperine, docking simulations, RNA polymerase.

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