Molecular interactions between NF-κB and thiopheneacetamide during mycobacteria infection

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The nuclear factor kappa B (NF-κB) pathway is a key role on the host response against many pathogens, as Mycobacterium tuberculosis, the etiological agent of tuberculosis (TB). TB is one of the oldest infectious disease in the world. After pathogen recognition by innate immune cells a serie of intracellular events are triggered culminating at the translocation of NF-κB to the nucleus. The DNA binding region of NF-kB is crucial for the coding of inflammatory genes resulting in the production of many inflammatory mediators implicated in the host defense against mycobacteria infection. In vitro studies showed that the tiophenolic compound, thiophenacetamide (TAA) and its analogs have moderate to low activity against M. tuberculosis and low cytotoxicity against macrophages. The aim of this study is to evaluate the binding mode of TAA and analogues within NF-κB, to study its dynamical behavior and the consequences in vitro of its interaction. These results will help us propose ways to interfere with the host immune response, in order to eliminate the infection. To achieve this goal, we used molecular docking and molecular dynamics methodologies for the in silico assays. For our in vitro studies we used an experimental model of macrophages infection. The binding pocket prediction retrieved eighteen possible cavities in the protein. Docking simulation recognized a particular pose for TAA and their analogues using AutoDock Vina 1.1.2 and DockThor server. TAA interacted mainly with Tyr57 and Val142 residues, as its analogues did with Tyr57, which composes of the NF-κB active site. The stability of cavities along time was checked, and variations in volume were detected. The ligand absence affected the average lifetime of the cavities. Molecular dynamics simulations revealed that the presence of DNA in the protein helps stabilize the complex (RMSD = 0,5Å). The simulation time was 50 nanoseconds using NAMD program. As our data in silico demonstrated a highly possibility of interaction between NF-κB and TAA, we analyzed whether TAA would have an immunomodulatory action on macrophages infected with M. bovis- BCG. It was observed in the supernatant of these cells a decreased in the release of TNF-α, IL-6. In addition a qualitative assessment of the nuclear translocation of NF-κB demonstrated that the TAA was able to inhibit it nuclear translocation. Molecular docking methodology suggested that TAA locates in cavities predicted by online servers. Also, molecular dynamics suggested that the DNA promotes stability to NF-κB while the absence of DNA promotes protein instability. In addition our in vitro results suggest a reduction on the inflammatory response caused by the infection.

Supported by: Fiocruz, Capes, FAPERJ.