Abstract

Introduction: Tuberculosis is a common infectious disease in the current century. Despite the high efficacy of isoniazid (INH), as an important first-line anti-TB drug, resistance toward INH is one of the serious challenges. Therefore, there is an urgent requirement to develop new anti-TB drugs. The current study aimed to study the stability of Mtb KatG mutants and suggest the appropriate isoniazid derivatives with capability to overwhelm disturbed binding pattern of INH toward S315T-KatG.

Methodology: Variants of KatG (Asp137, Ser315 & Met255) were all constructed through virtual mutagenesis. The stability and functionality variations of KatG upon SPMs were assessed. Intermolecular binding interactions were evaluated by AutoDock4.2 software. Clinical KatG mutant forms and the highest destabilizing mutants were subjected to energy decomposition analysis via density functional theory (DFT) calculation using 6-31G* (d, p) basis set. In the next phase, based on the literature review, several isoniazid derivatives, with high potency against drug-sensitive (MIC≤7.8μM) and resistant Mtb were selected. Molecular docking of intended INH derivatives provided top-scored compounds (**5** and **7**). In the next step, molecular dynamics simulation was carried out on top-ranked complexes by Gromacs5.1.1 program to evaluate the stability and achieve persistent binding pattern. Finally, the simulated complexes were subjected to DFT analysis by Gaussian09 package.

Results: It was revealed that all Ser315 variants retained the stability of the KatG structure. The calculations showed that all damaging effects of Asp137 and Ser315 variants occurred as the results of expansion or contraction of the cavity volume. With regard to $\Delta\Delta G_{mutation}$, no significant change in binding of INH to S315T could be estimated. MD trajectories were used to estimate average binding free energies for the top-scored complexes (Complexes of **5** to WT-KatG and S315T-KatG: -13.78±2.5 and -15.62±2.34, and Complexes of **7** to WT-KatG and S315T-KatG: -10.56±1.91 and -13.50±2.33 kcal/mol).

Discussion & conclusion: Variations in Gibbs free energy of folding were relatively correlated to corresponding entropy changes of KatG mutations. The results of molecular docking confirmed the importance of the imine bond in the structure of isoniazid derivatives and compounds **5** (N'-cyclopentylideneisonicotinohydrazide) and **7** (N'-(E) -(4-phenoxybenzylidene) isonicotinohydrazide) were selected as top-scoring molecules. Molecular docking & molecular dynamics studies revealed two derivatives **5** & **7** with higher capability of binding to S315T-KatG with regard to WT-KatG. The results of the current study may provide an appropriate structural basis to design new INH derivatives with potential drug-resistance overwheliming effect and develop new MDR-TB agents.

Keywords: Tuberculosis, KatG, Isoniazid, Molecular dynamics, SBR