Mycobacterial Diseases
ISSN: 2161-1068

Jena et al., Mycobact Dis 2016, 6:1
DOI: 10.4172/2161-1068.1000202

Commentary open access journal

Computational Approach in Understanding Mechanism of Action of Isoniazid and Drug Resistance

Jena L, Wankhade G, Waghmare P and Harinath BC*

Department of Bioinformatics, Tropical Disease Research Centre, India

***Corresponding author:** B.C. Harinath, JB Tropical Disease Research Centre, Mahatma Gandhi Institute of Medical Sciences, Sevagram - Wardha, Maharashtra, India, Tel: +91 7152 – 284341; E-mail: bc_harinath@yahoo.com

Received date: February 24, 2016; **Accepted date:** March 9, 2016; **Published date:** March 22, 2016

Copyright: © 2016 Jena L, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Most Multi Drug Resistance and Extremely Drug Resistance clinical strains of *Mycobacterium tuberculosis* are found to be resistant to the anti-tuberculousis drugs such as Isoniazid and Rifampicin. The mechanism of action and drug resistance due to Isoniazid has been the subject of extensive study. According to Tuberculosis drug resistance mutation database, 22 genes/proteins are associated with Isoniazid resistance such as katG, nat, inhA, ahpc, ndh, kasA etc. Mutation in the gene seems to affect the formation of Isoniazid to its active form or enhancing the catabolism thus making it ineffective. Studies in different laboratories have shown usefulness of computational approach in elucidating the mechanism of action of Isoniazid and development of drug resistance. Computational studies in our laboratory showed that a mutation in KatG (S315T/S315N) prevents free radical formation, thus the development of resistance to the drug. Further, we observed through molecular dynamics simulation approach that mutation (G67R/G207E) in NAT enzyme increases the stability and catalytic ability of the mutant enzyme, thus making the drug ineffective.

Keywords Mortality rate; Drug resistance; Bioinformatics; Computational approach

Introduction

Mortality rate and cases of Multi Drug Resistance (MDR) and Extremely Drug Resistance (XDR) are increasing day by day in case of tuberculosis for various reasons. Tuberculosis is the second leading cause of death followed by HIV. Most MDR and XDR clinical strains of MTB are found to be resistant to the anti-tuberculousis drugs such as Isoniazid (INH) and Rifampicin (RIF). Understanding the mechanism of action of these drugs like Isoniazid and Rifampicin and insight into drug resistance will help in developing more effective drugs for tuberculosis treatment. Studies in different laboratories have shown usefulness of computational approach to understand the mechanism of action of isoniazid and development of drug resistance.

The mechanism of action of INH has been the subject of rigorous studies. It is reported to generate a variety of highly reactive compounds, including reactive oxygen species (ROS) such as superoxide, peroxide and hydroxyl radical [1], nitric oxide [2], reactive organic species such as isonicotinic acyl radical or anion [3], and certain electrophilic species [4], which then attack multiple targets in MTB [5,6]. INH passively diffuses through the mycobacterial envelope; this prodrug is activated by $MnCl_2$ and the KatG, possibly into an isonicotinic acyl radical or anion. InhA enzyme is the major target in the mode of action of isoniazid, that needs activation to form the InhA inhibitory INH-NAD adduct [7]. Computational study of the binding of tautomer forms of INH–NAD adducts to the active site of InhA revealed that the 4S-chain adduct is effective active form of INH–NAD adducts [8]. Mahapatra et al. [9] in their mass spectrometry study isolated a novel metabolite 4-isonicotinoylnicotinamide (4-INN), a truncated part of the INH–NAD adduct from urine samples of human TB patients who received INH therapy. The truncated INH–NAD+ adduct with some structural similarity to 4-INN was evaluated as an effective inhibitor of InhA [10]. Our previous *in silico* docking study revealed that the INH–NAD adduct, which is generated *in vivo* after INH activation, may undergo spontaneous hydrolysis to form the truncated INH–NAD adduct and further binds and inhibits multiple enzymes of MTB, in addition to InhA, confirming that INH is an effective anti-TB drug acting at multiple enzymes [11].

According to TB drug resistance mutation database, 22 genes/proteins are associated with INH resistance [12]. Few genes reported to be induced by presence of INH, like fbpC, efpA, fadE23, fadE24 and ahpC [13]. A link between the inhibition of InhA and the inhibition of mycolic acid synthesis is provided by the fact that a mutation in the inhA gene, which confers INH resistance, also leads to inhibition of mycolic acid biosynthesis by INH [14]. KatG, a catalase-peroxidase is a multifunctional enzyme of MTB, exhibiting catalase, broad-spectrum peroxidase activity and a peroxynitritase activity. KatG also plays a role in the intracellular survival of mycobacteria within macrophages, protecting against reactive oxygen and nitrogen intermediates produced by phagocytic cells [15]. Although various mutations in the katG gene have been reported in INH-resistant isolates, the single amino acid mutation at Ser315 of the katG gene is reported to be the most widespread mutation, associated with INH resistance [16]. Another mutation, S315N (AGC/AAC) also reported in INH resistance strain [17].

Computational studies in our laboratory showed that a mutation in KatG (S315T/S315N) prevents free radical formation, thus the development of resistance to the drug [18]. In this study, important mutations in KatG i.e., S315T and S315N were modelled to compare with wild type KatG to study the influence of mutation on INH. In case of wild type KatG, no H-bond formation occurred between S315 residue and INH that may lead to the formation of free radical. In contrast, the mutations in KatG at amino acid position S315T and S315N have shown hydrogen bond formation between INH with

mutant residues. This H-bond formation may hamper INH-derived free radical formation. In addition, docking analysis between INH and INH-NAD adduct with InhA showed that the INH-NAD adduct is more effectively inhibiting InhA, Jena et al. [11,18].

Further, another computational study observed decreased flexibility of binding site residues in KatG mutation (S315T) which hampers INH activity. Ramasubban et al. [19] in their structural bioinformatics study elucidated the molecular mechanism of isoniazid resistance in katG gene (His276Met, Gln295His and Ser315Thr) and found that there was decrease in the stability and flexibility of the mutant proteins. Another recent study reported 23 novel katG mutations causing isoniazid resistance in clinical MTB isolates [20]. Mutation in other several genes including inhA, ahpc, ndh and kasA were reported to associate with isoniazid resistance [21].

Interestingly, like KatG, arylamine N-acetyltransferase (NAT) of MTB directly interacts with INH. NAT acetylates INH by transferring an acetyl group from acetyl coenzyme A to the terminal nitrogen of the drug, which in its N-acetylated form i.e., N-acetylated INH, is therapeutically inactive [15]. The over expression of NAT in *Mycobacterium smegmatis* explains increased resistance to INH [22]. In addition, when the gene was knocked-out, the bacteria exhibited increased sensitivity to INH [23]. Therefore, it is likely that NAT competes with KatG for INH. Computational studies in our laboratory showed that mutation in NAT enzyme increases the stability and catalytic ability of the mutant enzyme, thus making the drug ineffective (unpublished data). Ramos et al. [24], also employed the computational techniques to investigate the binding of isoniazid to three TBNAT isoforms: wild type, G68R and L125M in order to reveal the possible mechanism of resistance.

The Alkyl hydroperoxide reductase C protein (AhpC) is an important protein of MTB involved in defense against oxidative stress and isoniazid (INH) resistance [25]. Certain mutations of the ahpC promoter region result in over expression of ahpC [26] as a compensatory mechanism for the loss of catalase activity due to KatG mutations [27] by maintaining the ability to defend against oxidative stress mediated through organic peroxides [25].

IniA gene involved in mycolic acid biosynthesis and efflux pump activity of MTB confers resistance to INH and Ethambutal (EMB) [28]. MTB lacking the iniA gene showed increased susceptibility to INH. The deletion of this gene also results in an accumulation of intracellular ethidium bromide, therefore suggests that iniA confers resistance to multiple drugs [29]. The over expression of jefA (Rv2459), a drug efflux gene of MTB, leads to increased resistance to EMB and INH in MTB via efflux pump like mechanism and contributes in the development of resistance to these drugs [30]. KasA mutations produced an enzyme that had a diminished catalytic activity but conferred enhanced resistance to isoniazid. *In vivo* analysis confirmed that over expression of each of the four mutants of KasA gene enhanced isoniazid resistance when compared to over expression of wild-type KasA [31].

Though, INH resistance has been reported to associate with so many genes/proteins of MTB, Isoniazid Preventive Therapy (IPT) is increasingly being recommended by WHO in healthy children and latent TB cases in HIV patients. Other than known resistance risk, INH therapy is also associated with side effects including toxicity, acute liver failure and induced tenosynovitis and further predicted risk of development of resistance with extensive use of INH. This fact needs to be considered before its widespread use [32]. Even though, there are extensive studies on INH resistance, the detailed molecular mechanism of resistance and induction in a number of proteins is yet to be thoroughly understood. Computational approach has been useful in elucidating mechanism of drug resistance.

References

1. Shoeb HA, Bowman BU, Ottolenghi AC, Merola AJ (1985) Peroxidase-mediated oxidation of isoniazid. Antimicrob Agents Chemother 27: 399-403.
2. Timmins GS, Master S, Rusnak F, Deretic V (2004) Nitric oxide generated from isoniazid activation by KatG: source of nitric oxide and activity against Mycobacterium tuberculosis. Antimicrob Agents Chemother 48: 3006-3009.
3. Rawat R, Whitty A, Tonge PJ (2003) The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the Mycobacterium tuberculosis enoyl reductase: adduct affinity and drug resistance. Proc Natl Acad Sci USA 100: 13881-13886.
4. Johnsson K, King D, Schultz P (1995) Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of tuberculosis. Journal of the American Chemical Society 117: 5009-5010.
5. Santos LC (2012) Review: the molecular basis of resistance in Mycobaterium tuberculosis. OJMM 2: 224-36.
6. Bernardes-Génisson V, Deraeve C, Chollet A, Bernadou J, Pratviel G (2013) Isoniazid: an update on the multiple mechanisms for a singular action. Curr Med Chem 20: 4370-4385.
7. Dias MV, Vasconcelos IB, Prado AM, Fadel V, Basso LA, et al. (2007) Crystallographic studies on the binding of isonicotinyl-NAD adduct to wild-type and isoniazid resistant 2-trans-enoyl-ACP (CoA) reductase from Mycobacterium tuberculosis. J Struct Biol 159: 369-380.
8. Stigliani JL, Arnaud P, Delaine T, Bernardes-Génisson V, Meunier B, et al. (2008) Binding of the tautomeric forms of isoniazid-NAD adducts to the active site of the Mycobacterium tuberculosis enoyl-ACP reductase (InhA): a theoretical approach. J Mol Graph Model 27: 536-545.
9. Mahapatra S, Woolhiser LK, Lenaerts AJ, Johnson JL, Eisenach KD, et al. (2012) A novel metabolite of antituberculosis therapy demonstrates host activation of isoniazid and formation of the isoniazid-NAD+ adduct. Antimicrob Agents Chemother 56: 28-35.
10. Delaine T, Bernardes-Génisson V, Quémard A, Constant P, Meunier B, et al. (2010) Development of isoniazid-NAD truncated adducts embedding a lipophilic fragment as potential bi-substrate InhA inhibitors and antimycobacterial agents. Eur J Med Chem 45: 4554-4561.
11. Jena L, Deshmukh S, Waghmare P, Kumar S, Harinath BC (2015) Study of mechanism of interaction of truncated isoniazid–nicotinamide adenine dinucleotide adduct against multiple enzymes of Mycobacterium tuberculosis by a computational approach. International Journal of Mycobacteriology 4: 276-283.
12. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, et al. (2009) Tuberculosis drug resistance mutation database. PLoS Med 6: e2.
13. Wilson M, DeRisi J, Kristensen HH, Imboden P, Rane S, et al. (1999) Exploring drug-induced alterations in gene expression in Mycobacterium tuberculosis by microarray hybridization. Proc Natl Acad Sci USA 96: 12833-12838.
14. Marrakchi H, Lanéelle G, Quémard A (2000) InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II. Microbiology 146: 289-296.
15. Sandy J, Holton S, Fullam E, Sim E, Noble M (2005) Binding of the anti-tubercular drug isoniazid to the arylamine N-acetyltransferase protein from Mycobacterium smegmatis. Protein Sci 14: 775-782.
16. Ramaswamy S, Musser JM (1998) Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuber Lung Dis 79: 3-29.
17. Haas WH, Schilke K, Brand J, Amthor B, Weyer K, et al. (1997) Molecular analysis of katG gene mutations in strains of Mycobacterium tuberculosis complex from Africa. Antimicrob Agents Chemother 41: 1601-1603.

18. Jena L, Waghmare P, Kashikar S, Kumar S, Harinath BC (2014) Computational approach to understanding the mechanism of action of isoniazid, an anti-TB drug. Int J Mycobacteriol 3: 276-282.
19. Ramasubban G, Therese KL, Vetrivel U, Sivashanmugam M, Rajan P, et al. (2011) Detection of novel coupled mutations in the katG gene (His276Met, Gln295His and Ser315Thr) in a multidrug-resistant Mycobacterium tuberculosis strain from Chennai, India, and insight into the molecular mechanism of isoniazid resistance using structural bioinformatics approaches. Int J Antimicrob Agents 37: 368-372.
20. Torres JN, Paul LV, Rodwell TC, Victor TC, Amallraja AM, et al. (2015) Novel katG mutations causing isoniazid resistance in clinical M. tuberculosis isolates. Emerg Microbes Infect 4: e42.
21. Zhang Y, Yew WW (2009) Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13: 1320-1330.
22. Payton M, Auty R, Delgoda R, Everett M, Sim E (1999) Cloning and characterization of arylamine N-acetyltransferase genes from Mycobacterium smegmatis and Mycobacterium tuberculosis: increased expression results in isoniazid resistance. J Bacteriol 181: 1343-1347.
23. Payton M, Gifford C, Schartau P, Hagemeier C, Mushtaq A, et al. (2001) Evidence towards the role of arylamine N-acetyltransferase in Mycobacterium smegmatis and development of a specific antiserum against the homologous enzyme of Mycobacterium tuberculosis. Microbiology 147: 3295-3302.
24. Ramos RM, Perez JM, Baptista LA, de Amorim HL (2012). Interaction of wild type, G68R and L125M isoforms of the arylamine-N-acetyltransferase from Mycobacterium tuberculosis with isoniazid: a computational study on a new possible mechanism of resistance. J Mol Model 18: 4013-4024.
25. Wilson T, de Lisle GW, Marcinkeviciene JA, Blanchard JS, Collins DM (1998) Antisense RNA to ahpC, an oxidative stress defence gene involved in isoniazid resistance, indicates that AhpC of Mycobacterium bovis has virulence properties. Microbiology 144: 2687-2695.
26. Zhang M, Yue J, Yang YP, Zhang HM, Lei JQ, et al. (2005) Detection of mutations associated with isoniazid resistance in Mycobacterium tuberculosis isolates from China. J Clin Microbiol 43: 5477-5482.
27. Kelley CL, Rouse DA, Morris SL (1997) Analysis of ahpC gene mutations in isoniazid-resistant clinical isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother 41: 2057-2058.
28. Colangeli R, Helb D, Sridharan S, Sun J, Varma-Basil M, et al. (2005) The Mycobacterium tuberculosis iniA gene is essential for activity of an efflux pump that confers drug tolerance to both isoniazid and ethambutol. Mol Microbiol 55: 1829-1840.
29. Louw GE, Warren RM, Gey van Pittius NC, McEvoy CR, Van Helden PD, et al. (2009) A balancing act: efflux/influx in mycobacterial drug resistance. Antimicrob Agents Chemother 53: 3181-3189.
30. Gupta AK, Reddy VP, Lavania M, Chauhan DS, Venkatesan K, et al. (2010) jefA (Rv2459), a drug efflux gene in Mycobacterium tuberculosis confers resistance to isoniazid & ethambutol. Indian J Med Res 132: 176-188.
31. Slayden RA, Barry CE (2002) The role of KasA and KasB in the biosynthesis of meromycolic acids and isoniazid resistance in Mycobacterium tuberculosis. Tuberculosis (Edinb) 82: 149-160.
32. Jena L, Harinath BC (2015) Efficacy and safety of isoniazid preventive therapy in light of increasing multi-drug resistance in tuberculosis. Int J Mycobacteriol 4: 354-355.