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Short Communication

Isoniazid, Mechanism of Action, Biological Activity, Resistance and Biotransformation

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ABSTRACT

Isoniazid is a synthetic antimicrobial and one of the most essential first-line drugs used in the treatment of tuberculosis. In addition, isoniazid has been used as a prophylactic drug for patients with latent *Mycobacterium tuberculosis* infection to prevent the reactivation of the disease. It is a prodrug that is activated by catalase-peroxidase (KatG) enzyme. Catalase peroxidase enzyme converts isoniazid to reactive species. Isoniazid reactive species inhibit enoyl acyl-carrier-protein reductase (InhA) enzyme, which is involved in the biosynthesis of fatty acids of mycobacteria. Isoniazid is metabolized by amidase enzyme into isonicotinic acid and hydrazine.
Chronic toxicity of isoniazid results in hepatotoxicity and peripheral neuropathy. For active tuberculosis, isoniazid is often used together with rifampicin, pyrazinamide, and either streptomycin or ethambutol. Multiple extensively and totally drug-resistant strains of *Mycobacterium tuberculosis* were reported. Due to the development of *Mycobacterium tuberculosis* resistance to isoniazid, a continuous search for new drugs is a demand to combat this global problem.

Keywords: Biological Activity, Isoniazid, Microbial Transformation, Resistance, Toxicity.

1. INTRODUCTION

Isoniazid (isonicotinic acid hydrazide) is one of the most common antituberculosis drugs. It is a prodrug, activated through an oxidation reaction catalyzed by catalase-peroxidase (KatG) enzyme, which converts isoniazid to reactive species.[1] Isoniazid reactive species bind to enoyl acyl-carrier-protein reductase (InhA) enzyme, which is involved in the biosynthesis of fatty acids. It forms a covalent complex with the NAD^+ cofactor of the enzyme and thus inhibits the synthesis of lipids and DNA, leading to the prevention of cell wall synthesis and development.[2, 3] First, isoniazid was recommended as a monotherapy for patients with latent tuberculosis. Then, it was frequently used in combination with pyrazinamide, rifampin, or both to combat antimicrobial resistance[4].

2. Synthesis of isoniazid

Several methods are available for the synthesis of isoniazid. Isoniazid is commercially synthesized using 4-cyanopyridine and hydrazine hydrate.[5] It is also synthesized from citric acid as a starting material.[6]

Furthermore, enzymatic synthesis uses lipases in a non-aqueous medium starting from ethyl isonicotinate and hydrazine (Figure 1). Lipases are well known to act as catalysts for esterification and trans-esterification reactions in non-aqueous media.[7]

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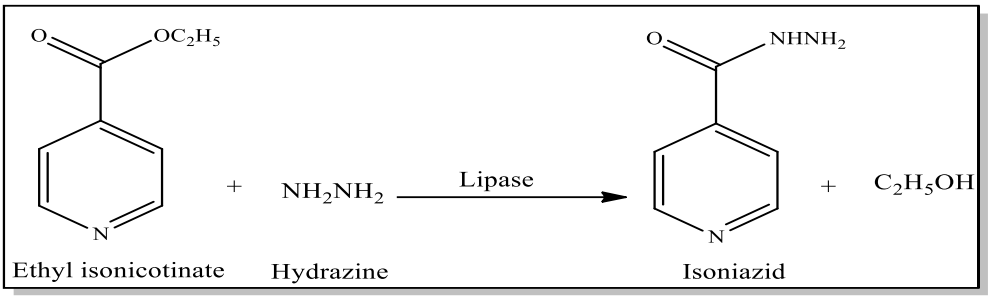

Figure 1: Enzymatic synthesis of isoniazid using lipases in non-aqueous media.

3. Biological activity and mechanism of action of isoniazid

Isoniazid is a prodrug that must be activated by bacterial catalase-peroxidase enzyme. Activation is associated with the reduction of the mycobacterial ferric catalase-peroxidase (KatG) enzyme by hydrazine moiety of isoniazid and reaction with oxygen to form an oxyferrous enzyme complex. Once activated, isoniazid prevents the synthesis of mycolic acids, an essential constituent of the bacterial cell wall. At therapeutic levels, isoniazid is bactericidal against actively growing intracellular and extracellular *Mycobacterium tuberculosis* organisms. Isoniazid inhibits InhA, the enoyl acyl reductase enzyme from *M. tuberculosis*, by forming a covalent adduct with the NAD cofactor. The isoniazid-NAD complex acts as a slow, tight-binding competitive inhibitor of InhA.[8]

4. Structure-activity relationship of isoniazid

The hydrazine moiety is important for the antituberculosis activity of isoniazid. By substituting the hydrazine moiety ($NHNH_2$) of isoniazid with an alkyl group, a range of active and inactive compounds appeared. Substitution on the terminal nitrogen gave active derivatives (R_1 = R_2 = alkyl, R_3 = H). Replacement of the amide nitrogen with alkyl groups decreased the activity.[9] It was also reported that the activity was abolished by isomerization of the pyridine nitrogen to other positions or its removal. Substitution of position 2 with a methyl group revealed antitubercular activity comparable to isoniazid (Figure 2).[10,11]

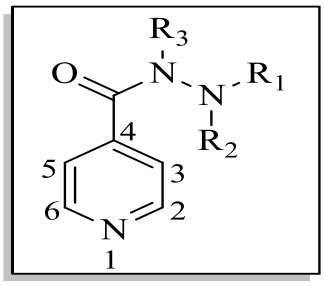

Figure 2: Structure-activity relationship of isoniazid.

It was found that some quinoxaline 1,4-di-*N*-oxide derivatives containing isoniazid as a pharmacophore (Figure 3) showed growth inhibition values of 99% and 100% against *Mycobacterium tuberculosis* H37Rv. In addition, it was reported that the absence of the two *N*-oxide groups generally led to the loss of the antitubercular activity.[12-14]

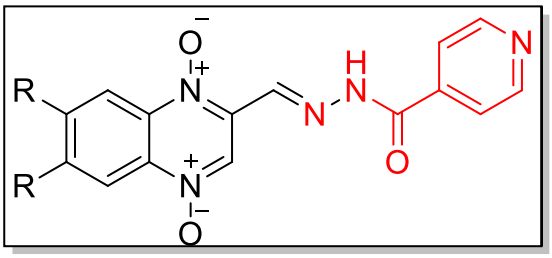

Figure 3: Structure of quinoxaline 1,4-di-*N*-oxide derivatives containing isoniazid as pharmacophore.

5. Resistance to isoniazid

Multiple and extensively drug-resistant strains of *M. tuberculosis* were reported. Multiple drug resistance (MDR) *M. tuberculosis* is resistant to at least rifampin and isoniazid (first-line antituberculosis drugs). Extensively drug resistance (XDR) *M. tuberculosis* is resistant to at least one fluoroquinolone and one or more second-line injectable antibiotics such as kanamycin, amikacin, and capreomycin.[15] A more worrying situation has appeared with the report of a totally drug-resistant strain which showed resistance to all antibiotics available for testing. [16] Early diagnosis of all forms of drug resistance in tuberculosis is critical for limiting and controlling the spread of these resistant strains. Understanding the mechanism of action and drug resistance of antituberculosis drugs can aid in the discovery of new drug targets. Two primary molecular pathways of *M. tuberculosis* resistance to isoniazid are linked to gene mutations in the KatG and InhA enzymes.[17]The most common gene mutation in the KatG enzyme is S315T (serine 315 mutation to threonine 315), which affects the activation of isoniazid. The second most common mutation is in the promoter region of the InhA enzyme, which induces InhA overexpression or, less commonly, a change in the active site.

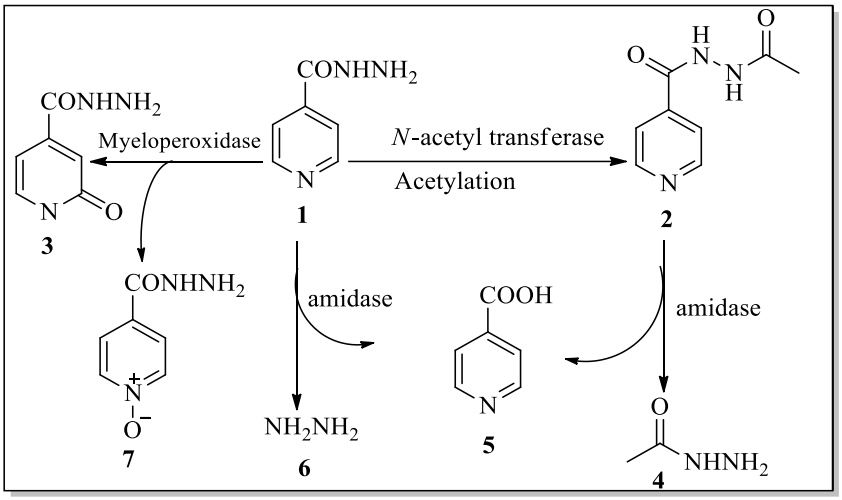

Figure 4: Mammalian metabolism of isoniazid. **1**: isoniazid, **2**: acetyl isoniazid, **3**: 2-oxo-1,2-dihydropyridine-4-carbohydrazide, **4**: acetylhydrazide, **5**: isonicotinic acid, **6**: hydrazine and **7**: isoniazid *N*-oxide.

Therefore, InhA affinity to the isoniazid-NAD complex is reduced.[18]

6. Isoniazid toxicity

Isoniazid treatment has a risk of toxicity which may be acute or chronic. Consumption of 2 gm of isoniazid causes acute toxicity, which appears as neurological symptoms. Chronic toxicity results in hepatotoxicity and peripheral neuropathy.[19,20]

7. Metabolism of isoniazid

7.1. Mammalian metabolism of isoniazid

Isoniazid is metabolized by the amidase enzyme into isonicotinic acid and hydrazine (Figure 4). Isoniazid is also converted to isoniazid *N*-oxide and 2-oxo-1,2-dihydropyridine-4-carbohydrazide by myeloperoxidase enzyme. It is also converted by *N*-acetyltransferase to acetyl isoniazid, which is metabolized by amidase enzyme to acetylhydrazine and isonicotinic acid.[20]

7.2. Microbial transformation of isoniazid

Isoniazid was converted to isonicotinamide by Mycobacterium bovis (Figure 5).[21] On the other hand, isoniazid was metabolized by Sarcina species into isonicotinic acid, which was further converted to 2-oxoisonicotinic acid followed by ring cleavage to finally generate pyruvate (Figure 6).[22] Moreover, a new study by Ragab et al. reported that three major metabolites 1-3 derived from isoniazid transformation by Aspergillus niger NRRL 328 and Aspergillus niger AUMC 4156. The metabolites were identified as isonicotinic acid, isonicotinic acid *N*-oxide, and isonicotinamide, respectively, as illustrated in Figure 7.[23] Antituberculosis activity of isoniazid and metabolites **1-3** against drug-sensitive, multiple drug resistance (MDR) and extensive drug resistance (XDR) were evaluated using microplate alamar blue assay. Isoniazid was used as a reference. The results revealed that, among the tested metabolites, metabolite **2** (isonicotinic acid *N*-oxide) was highly active against the drug-sensitive Mycobacterium tuberculosis strain ATCC 25177/ H37Ra. The obtained results also revealed that metabolite **2** was four-fold more active than isoniazid. Furthermore, metabolite **2** (isonicotinic acid *N*-oxide) was more active than metabolite **3** (isonicotinamide) against multiple drug-resistant strains ATCC 35822. Metabolite **2** was the only active metabolite against an extensively drug-resistant strain, a clinical isolate from the Regonal Center of Microbiology and Biotechnology (RCMB). The effect of metabolite **2** on the inhibition of M. tuberculosis InhA enzyme was determined, and the study revealed that metabolite **2** was a more potent inhibitor of InhA enzyme with IC50 0.20±1.20 µM compared to isoniazid IC50 0.70±0.58 µM. The InhA enzyme assay results comply with that obtained from the determination of the MIC value of metabolite 2. Therefore, metabolite **2** (isonicotinic acid *N*-oxide) is a potent antituberculosis agent targeting InhA enzyme. The antituberculosis activity was confirmed by good protein-ligand binding affinity to InhA enzyme using MVD software which revealed that metabolite **2** with the *N*-oxide structure showed the highest affinity of binding to InhA active site (MolDock score -73.44 kcal/mol) compared to isoniazid (MolDock score -71.38 kcal/mol). This is in concordance with the observed in vitro InhA enzyme assay results.

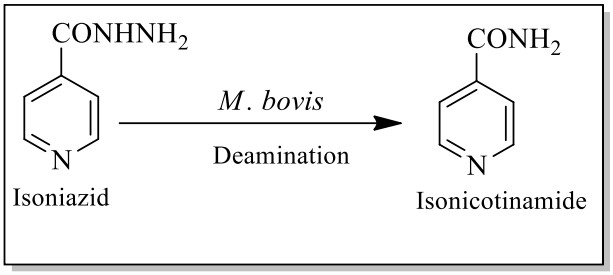

Figure 5: Metabolism of isoniazid by *M. bovis.*

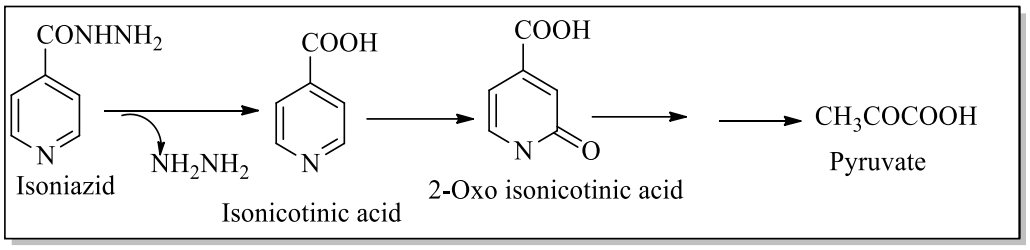

Figure 6: Isoniazid metabolism by Sarcina spices.

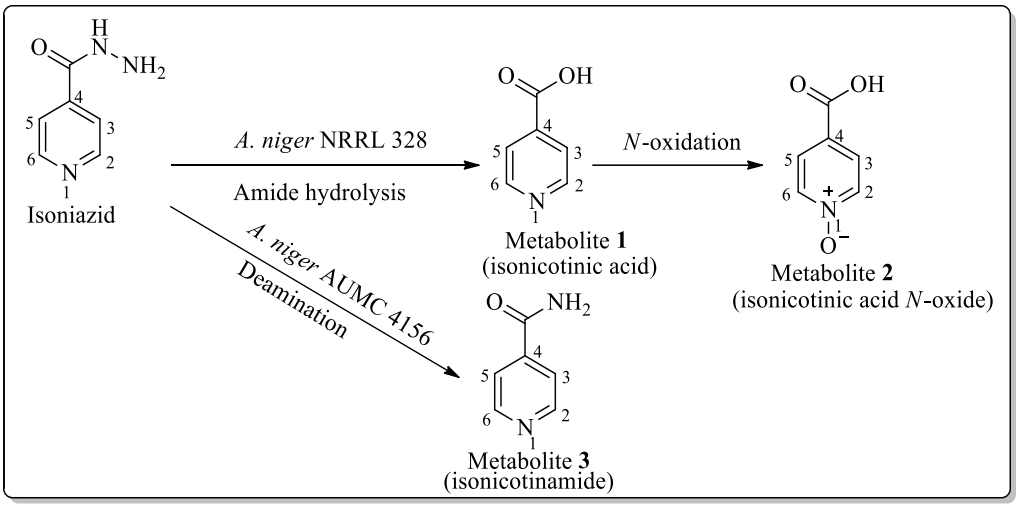

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Figure 7: Microbial transformation of isoniazid by *A. niger* NRRL 328 and *A. niger* AUMC 4156.

8. CONCLUSION

Isoniazid is still regarded as one of the most important drugs for the treatment of tuberculosis. Its efficacy is mostly due to its high potency and selectivity against Mycobacteria. This review aims to provide an overview of the biotransformation of isoniazid and the relative biological activity of the isolated metabolites against drug-resistant strains of Mycobacteria strains.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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