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Isoniazid in Tuberculosis Management: A Comparative Study With other First Line Drugs

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Abstract

The primary cause of tuberculosis (TB), Mycobacterium tuberculosis, continues to be a serious global health concern, especially in developing nations. Timely diagnosis and a combined medication therapy approach are essential for effective TB care. The combination of isoniazid, rifampicin, pyrazinamide, and ethambutol in the first-line anti-TB regimen works in concert to eradicate the infection and stop resistance. With a broad tissue distribution, including the central nervous system, good oral bioavailability, and renal clearance, the medication has favorable pharmacokinetics. The pyridine ring and hydrazide group are crucial to its anti-mycobacterial action, according to structure-activity relationship (SAR) studies. Disparities in the structure, mode of action, pharmacological characteristics, and formulations of first-line TB medications are revealed by comparative study.

Various in vitro and in vivo methods are employed in TB research, including drug susceptibility testing, molecular diagnostics, and animal models for efficacy evaluation. Together, these tools aid in understanding drug action, resistance mechanisms, and therapeutic responses.

INTRODUCTION

One infectious disease that can infect your lungs or other tissues is tuberculosis. Although it usually affects the lungs, it can also damage the spine, brain, or kidneys. A Latin term for "nodule" or something protruding is the source of the word "tuberculosis." Tuberculosis is sometimes referred to as TB. In 2020, around 1.5 million people worldwide lost their lives to tuberculosis, and over 10 million people contracted the disease. A communicable infection, tuberculosis (TB) typically affects the lungs. It may spread to other areas of your body, such as your spine and brain. Mycobacterium tuberculosis is the type of bacteria that causes it.

The infectious disease tuberculosis is brought on by a number of mycobacterium species. It spreads quickly, either directly or indirectly, from one person to another. The World Health Organization states that mycobacterium tuberculosis, the organism that causes tuberculosis, typically affects the lungs. It is possible to prevent and cure tuberculosis.

Mycobacterium tuberculosis, the main cause of tuberculosis (TB), is a major global health concern, particularly in developing countries. Effective treatment of tuberculosis Especially TYPES

• Primary tuberculosis: This condition develops in people who are initially exposed to Mycobacterium tuberculosis.

When immunity is compromised, progressive primary TB develops. It most frequently occurs in young children, teenagers, and the elderly.

• Post-primary tuberculosis: Usually occurs in adults as a result of exogenous reinfection or endogenous reactivation in a previously sensitized (infected and treated) patient who has maintained some level of acquired immunity.

Two forms of tuberculosis are observed depending on the site of infection, including: pulmonary tuberculosis and extrapulmonary tuberculosis.

SYMPTOMS

- Chest pain
- Coughing up blood
- Feeling tired all the time
- Night sweats
- Fever
- Loss of appetite
- Weight loss
- When TB is outside the lungs, you may have these same symptoms along with pain near the area that's infected.

RISK FACTOR

- A friend, co-worker, or family member has active TB.

- You work or live in a hospital or nursing home.
- You're a health care worker for patients at high risk of TB.
- You're a smoker.
- Low body weight and poor nutrition.
- Immunocompromised patients.

ETIOLOGY

o The most prevalent and significant agent causing human illness is Mycobacterium tuberculosis, one of the bacteria that cause TB, an infectious disease.
o M. bovis, M. africanum, and M. microti are closely related mycobacteria that can infrequently cause similar disease.
o Robert Koch won a noble prize in 1905 for his discovery that the tubercle bacillus was the actual cause of tuberculosis (TB), which he proved in 1882.

PATHOPHYSIOLOGY

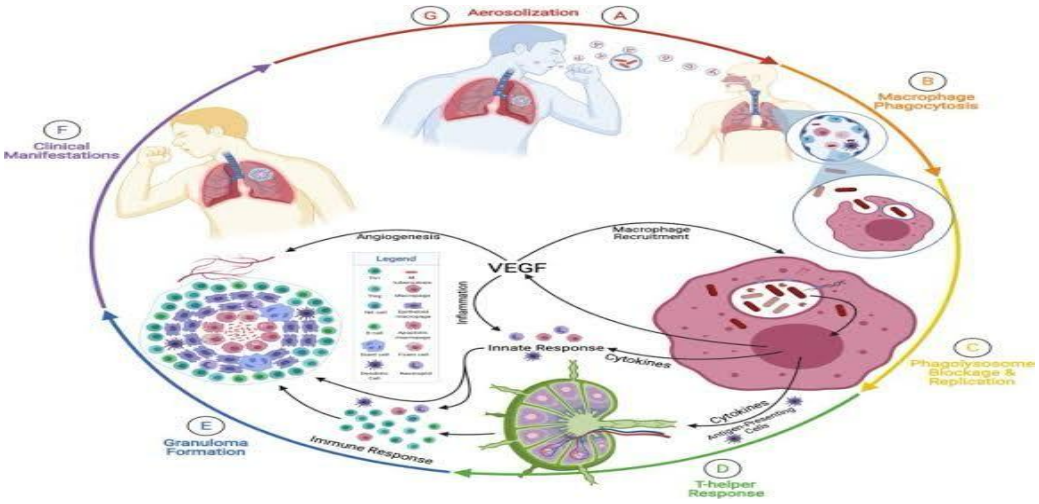


Fig .1 Pathophysiology of tuberculosis

The pathophysiology of tuberculosis, which is caused by Mycobacterium tuberculosis infections, is a complex interaction between physiological and pathogenic processes.
A) The pathophysiological cycle of tuberculosis starts and ends with aerosolization. When a person with active tuberculosis coughs or otherwise violently expires, aerosolization takes place.
B) A susceptible individual will come into contact with monocytes, dendritic cells, and macrophages if they inhale aerosolized Mycobacterium tuberculosis and droplets small enough to enter the alveolar sacs (shown in the first magnification). As seen in the second magnification, the macrophages will phagocytose the bacteria and try to eliminate the invader. T-helper cells will be activated by dendritic cells migrating to lymph nodes.
C) M. tuberculosis starts reproducing, releases DNA, RNA, proteases, and lipids, avoids destruction, and stops the phagolysosome fusion. The macrophages will also release vascular endothelial growth factor (VEGF) and cytokines. VEGF will promote vascularization to the lesion by inducing angiogenesis. The cytokines will trigger the innate response and attract various types of neutrophils, macrophages, dendritic cells, and natural killer cells.
D) TH1, Tregs, and B cells primed in the germinal center will migrate as part of the T-helper cell response. The granuloma will be formed by the combination of these cells.
E) By acting as a jail, the granuloma prevents the germs from spreading throughout the body.
F) The granuloma is unable to hold the bacteria due to immunological compromise that occurs later or now. In a variety of clinical symptoms, the bacteria will proliferate and spread.
G) In the course of this phase, the original susceptible, now infected host may aerosolize the germs, starting a new cycle.

DIAGNOSIS

Although TB can be diagnosed using a variety of tests, a medical professional will typically begin by using a stethoscope to listen to a patient's breathing and looking for enlarged lymph nodes. After that, they will probably perform some more tests to find out if a person has latent or active tuberculosis.

Blood tests

These tests, also known as interferon-gamma release assays (IGRAs), quantify the reaction to a little sample of your blood combined with TB proteins. Testing for blood can help identify or rule out latent or active tuberculosis. The tests assess how your body responds to the TB germs. For tuberculosis, the Food and Drug Administration has approved two blood tests:

- T-SPOT TB test (T-Spot)
- QuantiFERON-TB Gold In-Tube test (QFT-GIT).

If your skin test results are positive, you most likely have TB bacteria. But you could also get a false positive. If you've gotten a tuberculosis vaccine called Bacillus Calmette-Guerin (BCG), the test could say you have TB when you really don't. You can also get a false negative, saying that you don't have TB when you really do, if your infection is very new.

Imaging tests

Following a positive skin test, a healthcare provider may order a chest X-ray or CT scan. These tests produce images that may show changes in the lungs caused by active TB.

TREATMENT

NON-PHARMACOLOGICAL TREATMENT

- Adequate nutrition
- Medico social awareness
- Less crowded living conditions
- Avoid dust
- Avoid smoking

PHARMACOLOGICAL TREATMENT

TB treatment is effective. Worldwide, nearly 90% of cases of TB and 48% of cases of drug-resistant TB are cured. However, treatment is not quick or easy. The length of treatment and side effects from the drugs used pose huge problems for TB patients and for global efforts to tackle the disease. Find out more under Global TB challenges.

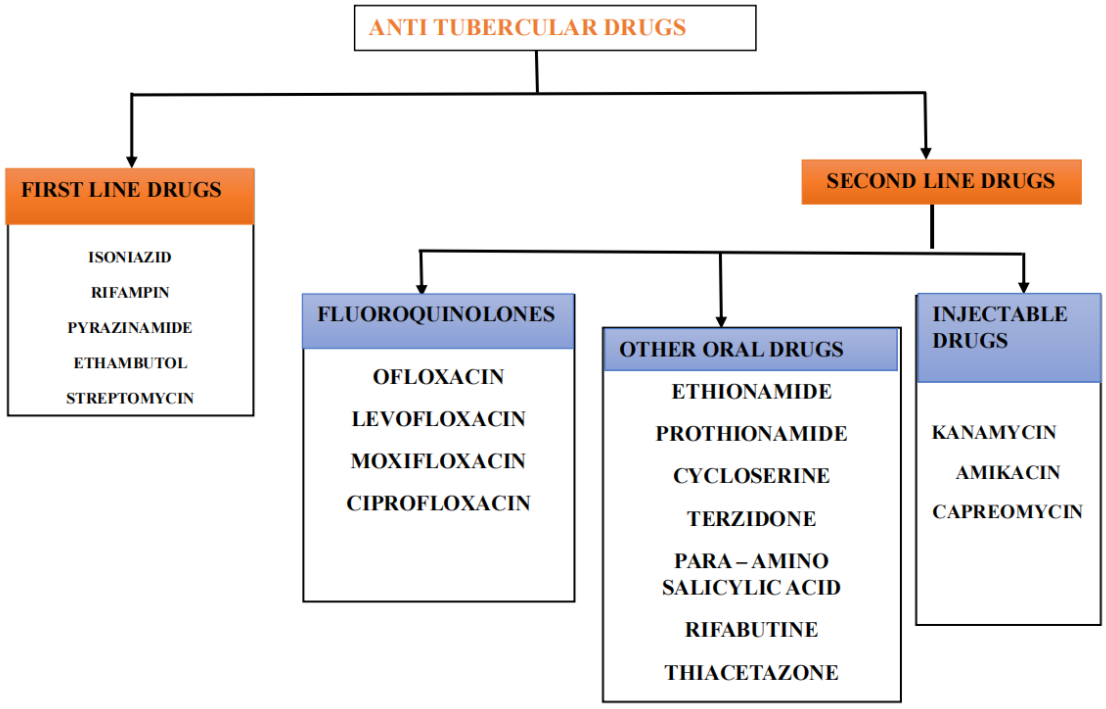


Fig.2 Classification of anti tubercular drug.

TB treatment lasts at least six months. Treatment for TB is usually a mixture of four antibiotics:

First Line:

- Rifampin10mg/kg-
- Isoniazid-5mg/kg
- Pyrazinamide-30-40mg/kg
- Ethambutol-15-25mg/kg

Second Line:

- Kanamycin (discontinued use in the USA)
- Streptomycin
- Capreomycin
- Amikacin

BCG vaccine

The primary purpose of the Bacillus Calmette–Guerin (BCG) vaccination is to prevent tuberculosis (TB). It bears Albert Calmette and Camille Guerin's names as its inventors. Healthy neonates should get one dosage as soon as possible after birth in nations where leprosy or tuberculosis are prevalent. Only children at high risk are usually vaccinated in regions where TB is uncommon. About 20% of youngsters are protected from infection by it, and half of those who are infected are shielded from illness. The vaccination is administered by skin injection.Serious negative effects are uncommon. The injection site frequently experiences redness, swelling, and slight pain.The vaccine was originally developed from Mycobacterium bovis, which is commonly found in cattle. While it has been weakened, it is still live.

ISONIAZID

When treating an active Mycobacterium tuberculosis (TB) infection, the antibiotic isoniazid (INH) is recommended as the first line of treatment. For many years, INH has been a key component of TB treatment plans. The catalase-peroxidase KatG activates INH, a prodrug that produces a variety of radicals and adducts that prevent the mycobacterium from producing mycolic acids, which are vital parts of its cell wall. INH is a strong bactericidal agent because of its mode of action. Additionally, INH works in concert with other species produced by KatG and other drugs used to treat tuberculosis.

Structure :

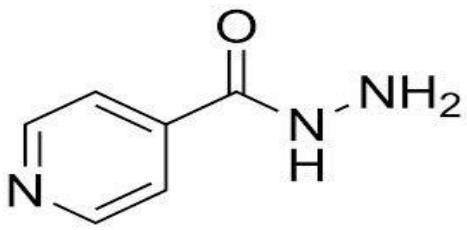


Fig.3 Structure of isoniazid

IUPAC : 4-Pyridinecarboxylic acid hydrazide or Pyridine-4-carbohydrazide.

SAR Of Isoniazid :

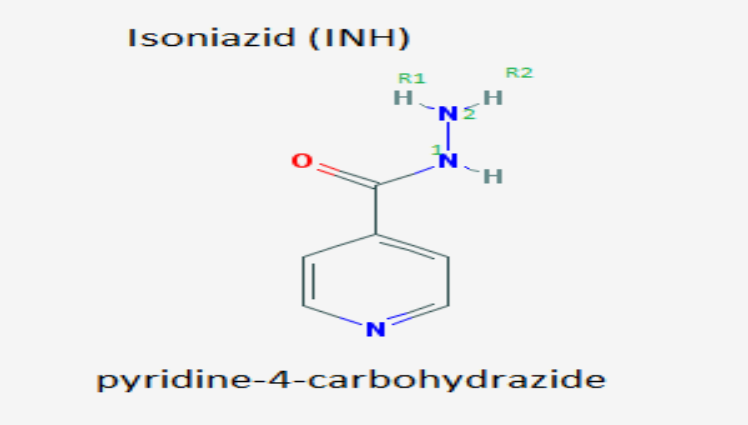


Fig.4 SAR of isoniazid

Pyridine ring is essential for activity, replacement of pyridine with benzene, thiazole, piperidine give loss of activity. Only 2-thiazole give some active agent.

- Position 4 of the ring is most suitable for substitution with carbohydrazine. Substitution at other positions will give less activity.
- Substitution of hydrogen at N_1 with alkyl group give inactive compound. So it should left unsubstituted.
- Substitution of H_2 and H_3 at N_2 with small alkyl groups give less activity or inactive compounds.
- Replacement of cycloalkyl, aryl alkyl groups of H_2 at N_2 will give active compounds.
- Example : Iproniazid (also have anti-depressive activity)

Mechanism of action :

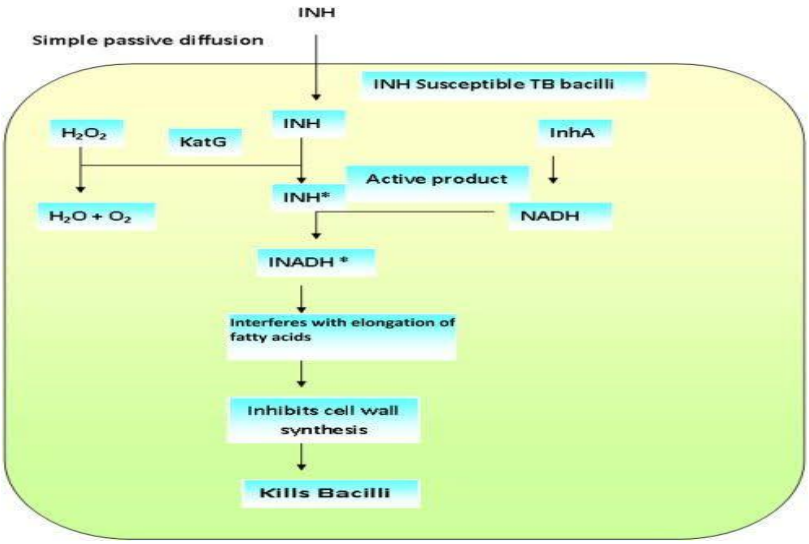


Fig.5 MOA of Isoniazid

PHYSICOCHEMICAL PROPERTIES

Molinspiration Cheminformatics Software

Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SD file conversion, normalization of molecules, generation of tautomers, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. Our products support also fragment-based virtual screening, bioactivity prediction and data visualization. Molinspiration tools are written in Java, therefore can be used practically on any computer platform.

Fig.6 Molinspiration Of Isoniazid

The physicochemical properties of isoniazid studies using molinspiration was found to be as –

PHYSICOCHEMICAL PROPERTY	VALUE
Mi LogP	-0.97
Number of atoms	10
Molecular Weight	137.14
Number of hydrogen bond donors	3
Number of hydrogen bond acceptors	4

Fig.7 Physicochemical properties of Isoniazid

LIPINSKI'S RULE OF FIVE

Lipinski's rule of five, also known as Pfizer's rule of five or simply the rule of five (RO5), is a rule of thumb to evaluate drug likeness or determine if a chemical compound with certain pharmacological or biological activity has chemical properties and physical properties that would likely make it an orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria;

• No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen– hydrogen bonds)

• No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)

• A molecular mass less than 500 daltons

• A calculated octanol-water partition coefficient (log P) that does not exceed 5

Since all the above conditions are satisfied by Isoniazid, it is considered to comply with Lipinski's Rule of Five.

ADMET Prediction:

Swiss ADME allows to compute physicochemical descriptors as well as predict pharmacokinetics properties and drug like nature of one or multiple small molecules.

The absorption, distribution, metabolism and excretion of isoniazid was assessed using the

Swiss ADME software.

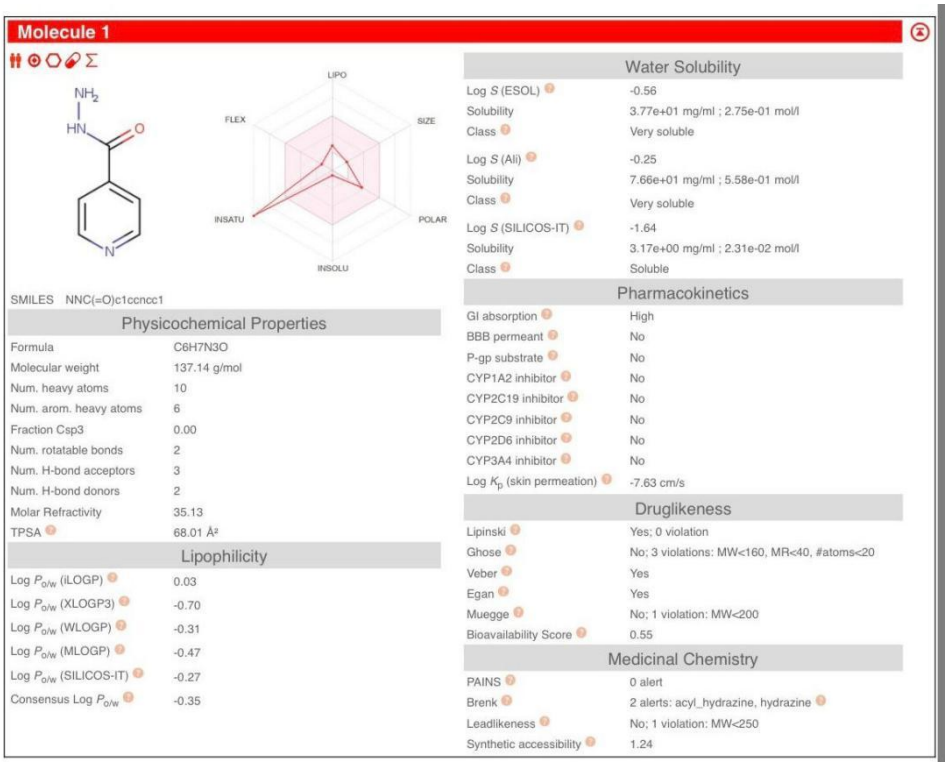


Fig.8 Swiss ADME of Isoniazid

Bioavailability Radar

Bioavailability Radar is displayed for a rapid appraisal of drug-likeness. Six physicochemical properties are taken into account: lipophilicity, size, polarity, solubility, flexibility and saturation. A physicochemical range on each axis was defined by descriptors and depicted as

a pink area.

Physicochemical property

The physicochemical property of Isoniazid was analysed using Swiss ADME and was

found to be;

PHYSICOCHEMICAL PROPERTY	VALUE
Molecular weight	137.14g/mol
Number of heavy atoms	10
Number of aromatic heavy atoms	6
Number of hydrogen bond donors	2
Number of hydrogen bond acceptors	3

Fig.9 Physicochemical Properties

Lipophilicity

The partition coefficient between n-octanol and water (log Po/w) is the classical descriptor for Lipophilicity. It has a dedicated section in Swiss ADME due to the critical importance of this physicochemical property for pharmacokinetics drug discovery. Swiss ADME give access to five freely available predictable models; which are XLOGP3, WLOGP, MLOGP, SILICOS-IT, iLOGP. The lipophilicity of Isoniazid was studied using Swiss ADME which determines the solubility, the ability to penetrate through cell barriers, and transport across the membrane.

LIPOPHILICITY	VALUE
LogPo/w(ILOGP)	0.03
LogPo/w(XLOGP3)	-0.70
LogPo/w(WLOGP)	-0.31
LogPo/w(MLOGP)	-0.47
LogPo/w(SILICOS-IT)	-0.27
ConsenusLogPo/w	-0.35

Fig.10 Lipophilicity of Isoniazid

Water solubility

soluble molecule greatly facilitates many drug development activities, primarily the ease of handling and formulation. Moreover, for discovery projects targeting oral administration, solubility is one major property influencing absorption. As well, a drug meant for parenteral usage has to be highly soluble in water to deliver a sufficient quantity of active ingredient in the small volume of such pharmaceutical dosage. Two topological methods to predict Water Solubility are included in Swiss ADME. The first one is an implementation of the ESOL model36 and the second one is adapted from Ali et al.

The water solubility of Isoniazid was studied using Swiss ADME.

Pharmacokinetics

Pharmacokinetics is essential for the knowledge about interaction of molecules with cytochromes P450 (CYP). This superfamily of isoenzymes is a key player in drug elimination through metabolic biotransformation. The pharmacokinetics of Isoniazid was studied using Swiss ADME. study shows that Isoniazid is not an inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4. Hence the drug do not possess any pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites.

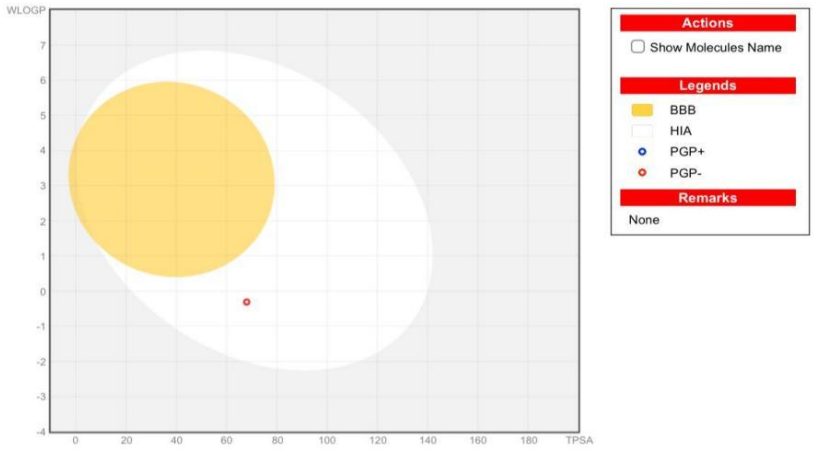


Fig.11 Pharmacokinetics of isoniazid

The boiled-egg allows for intuitive evaluation of passive gastrointestinal absorption (HIA) and brain penetration (BBB) in function of the position of the molecules in the WLOGP-versus-TPSA referential. In addition the points are coloured in blue if predicted as actively effluxed by P-gp (PGP+) and in red if predicted as non-substrate of P-gp (PGP−). In case Isoniazid is located in white region of boiled egg which shows that Isoniazid has high probability of passive absorption by the gastrointestinal tract. Isoniazid is not subject to active efflux by P-gp. (PGP+(red dot)).

SYNTHESIS

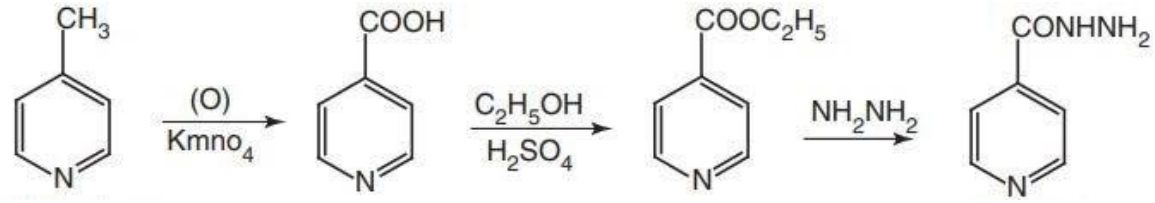


Fig.12 Synthesis of Isoniazid

4-Pycoline Isonicotinic acid Ethyl isonicotinate Isoniazid

Isoniazid is an isonicotinic acid derivative. It is manufactured using 4-cyanopyridine and hydrazine hydrate. In another method, isoniazid was claimed to have been made from citric acid starting material. It can in theory be made from methyl isonicotinate, which is labelled a semi chemical.

ASSAY OF ISONICOTINIC ACID HYDRAZIDE TABLET

Twenty tablets were weighed accurately and pulverized. A weighed quantity of the tablet power equivalent to 0.25 mg INH was transferred into a clean and dry 100 ml volumetric flask, the sufficient water was added to produce 100 ml. 20 ml of above solution was taken. Then 100 ml of water, 20ml of hydrochloric acid and 0.2 gm of Potassium bromide were added. Then titrated against slowly with continuous shaking with 0.0167M potassium bromate using 0.05ml of methyl red as an indicator until red colour disappears

Each ml of 0.0167M Potassium bromate KBr03 = 0.003439g of C6H7N3O.

COMPARITIVE STUDY OF FIRST LINE DRUGS

STRUCTURAL COMPARISON

Mechanisms of Action and Efficacy

- **Isoniazid**: Inhibits the synthesis of mycolic acids, essential components of the mycobacterial cell wall. It is highly effective against rapidly dividing Mycobacterium tuberculosis and is often used in combination with other drugs to prevent resistance development.
- **Rifampin**: Binds to the β-subunit of bacterial RNA polymerase, inhibiting RNA synthesis. It exhibits potent bactericidal activity and is crucial for sterilizing infections. Rifampin is effective against both actively dividing and semi-dormant bacilli.

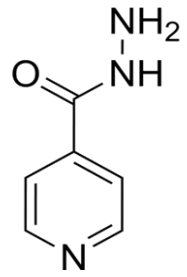
- **Pyrazinamide**: Converted to pyrazinoic acid in the acidic environment of macrophages, disrupting the bacterial membrane potential. It is particularly effective against semi-dormant bacilli and contributes to shortening the treatment duration.
- **Ethambutol**: Inhibits arabinosyl transferases involved in the synthesis of arabinogalactan, a component of the mycobacterial cell wall. While bacteriostatic, it is included in treatment regimens to prevent the development of resistance.
- **Streptomycin**: An aminoglycoside that binds to the 30S ribosomal subunit, inhibiting protein synthesis. It is effective in severe or extrapulmonary tuberculosis but is less commonly used due to its toxicity profile.

Safety Profiles

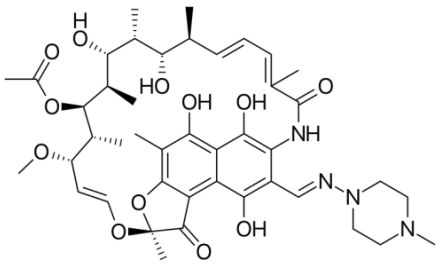
- **Isoniazid**: Associated with hepatotoxicity, especially in older adults. Peripheral neuropathy can occur, which is often prevented with pyridoxine supplementation.
- **Rifampin**: Can cause hepatotoxicity and induces cytochrome P450 enzymes, leading to potential drug interactions. It also imparts an orange-red discoloration to bodily fluids.
- **Pyrazinamide**: Hepatotoxicity is a significant concern, particularly at higher doses. It can also increase serum uric acid levels, potentially leading to gout.
- **Ethambutol**: The primary adverse effect is optic neuritis, which can impair vision and is dose-related. Regular eye examinations are recommended during treatment.
- **Streptomycin**: Ototoxicity and nephrotoxicity are major concerns, limiting its use in current regimens.

Pharmacokinetics

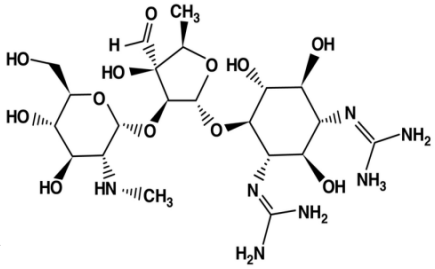
- **Isoniazid**: Rapidly absorbed from the gastrointestinal tract and widely distributed throughout the body. It is metabolized in the liver and excreted in the urine.
- **Rifampin**: Well absorbed and widely distributed. It induces cytochrome P450 enzymes, affecting the metabolism of other drugs.
- **Pyrazinamide**: Rapidly absorbed and distributed throughout the body. It is metabolized in the liver and excreted in the urine.
- **Ethambutol**: Well absorbed and widely distributed. It is excreted unchanged in the urine.
- **Streptomycin**: Poorly absorbed orally and is administered parenterally. It is excreted unchanged in the urine.



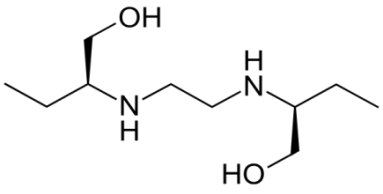
Isoniazid



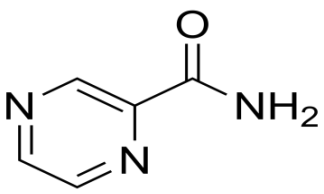
Rifampin



Streptomycin



Ethambutol



Pyrazinamide

Fig.13 Structures of Isoniazid, Rifampin ,Streptomycin, Ethambutol, Pyrazinamide

COMPARITIVE SAR OF FIRST LINE DRUGS

1. ISONIAZID (INH)

- **Chemical Structure**: A pyridine ring (nicotinic acid) attached to a hydrazide group (-NH-NH_2).
- **Mechanism of Action**: Activated by the bacterial enzyme KatG to form a reactive radical that inhibits mycolic acid synthesis via binding to NADH-dependent enoyl-acyl carrier protein reductase (InhA).
- **Structural Significance**: The hydrazide group is crucial for its activity; modifications can abolish activity.

2. RIFAMPIN

- **Chemical Structure**: A complex macrocyclic structure with a naphthalene ring system and a polyketide backbone.
- **Mechanism of Action**: Binds to the β-subunit of bacterial RNA polymerase, inhibiting RNA synthesis.
- **Structural Significance**: The naphthalene ring system is essential for binding; modifications can affect binding affinity and spectrum of activity.

3. PYRAZINAMIDE

- **Chemical Structure**: A pyrazine ring with a carboxamide group.
- **Mechanism of Action**: Converted to pyrazinoic acid by pyrazinamidase; the active form disrupts bacterial membrane potential in acidic environments.
- **Structural Significance**: The pyrazine ring is vital; substitutions can reduce activity.

4.ETHAMBUTOL

- **Chemical Structure**: An aliphatic chain with two chiral centers.
- **Mechanism of Action**: Inhibits arabinosyl transferases, disrupting arabinogalactan synthesis in the mycobacterial cell wall.
- **Structural Significance**: Chirality is crucial; racemic mixtures or incorrect isomers have reduced or no activity.

5.STREPTOMYCIN

- **Chemical Structure**: An aminoglycoside with multiple amino sugars.
- **Mechanism of Action**: Binds to the 30S ribosomal subunit, inhibiting protein synthesis.
- **Structural Significance**: The aminoglycoside structure is essential for binding and activity; modifications can affect binding affinity and resistance profiles.

PHARMACOKINETIC PROPERTY

CLASS	BIOAVAILABILITY (%)	HALF-LIFE (hours)	CYP METABOLISM	P-GROUP SUBSTRATE	BBB PENETRATION
ISONIAZID	~90-95	• Fast acetylors-0.5-1.6 • Slow acetylors-2-5	minimal effect on CYP3A4	No	Yes
RIFAMPIN	~90-95	1.5–5 hours	CYP3A4	No	No
STREPTOMYCIN	~1	• Adults with normal renal function: ~ 2–3hr • Elderly individuals:~9hr • Premature/newborn infants:~ 4–10hr • Adults with severe renal impairment :~ 50–110hr	No CYP interaction	No	No
ETHAMBUTOL	~75-80	3–4 hours	Minimal CYP involvement	No	No
PYRAZINAMIDE	~90	~ 10 hours	Minimal CYP involvement	No	Yes

Fig.14 Comparative Study of Physicochemical properties

PHARMACOKINETIC PROPERTY

CLASS	MOLECULR WEIGHT (g/mol)	LogP (Lipophilicity)	TPSA	ROTATABLE BONDS	HYDROPHILICITY
Isoniazid	137.14	~ -0.69	68.01	1	HIGH
Rifampin	822.95	~ 2.7	223.64	5	MODERATE
Streptomycin	581.58	~ -2.53	383.00	9	VERY HIGH
Ethambutol	204.81	~ 0.4	64.53	9	MODERATE
Pyrazinamide	123.11	~ -0.60	68.88	1	HIGH

Fig.15 Comparative Study of Pharmacokinetic Properties

FIRST LINE DRUGS DOSAGE FORM AVAILAIBILITY Vs. MARKET AVAILABILITY

1. ISONIAZID (INH)

- **Dosage Forms**: Tablets (100 mg, 300 mg), Oral Syrup (10 mg/mL).
- **Market Availability**: Widely available in both public and private sectors. Fixed-dose combinations (FDCs) with Rifampin, Pyrazinamide, and Ethambutol are commonly used in India.

2. ETHAMBUTOL

- **Dosage Forms**: Tablets (100 mg, 400 mg), Oral Syrup.
- **Market Availability**: Available in the Indian market; however, there have been reports of shortages, particularly for the 100 mg and 400 mg tablet strengths.

3. STREPTOMYCIN

- **Dosage Forms**: Injectable (1 g vial).
- **Market Availability**: Available in India, primarily used in drug-resistant TB cases.

4. PYRAZINAMIDE

- **Dosage Forms**: Tablets (500 mg), Oral Suspension.
- **Market Availability**: Widely available in India. Tablets are commonly used, while oral suspensions are available for pediatric patients or those who have difficulty swallowing tablets.

*5. **RIFAMPIN***

- **Dosage Forms**: Capsules (150 mg, 300 mg), Oral Suspension (20 mg/mL), Injectable (600 mg vial).
- **Market Availability**: Rifampin is available in India in various forms. However, there have been reports of shortages for certain formulations, such as the 150 mg and 300 mg oral tablets and the oral liquid form.

SUMMARY OF CLINICAL USE BY POTENCY

1. ISONIAZID (INH)

- **Active Tuberculosis**: Isoniazid is a cornerstone in the treatment of active TB, typically administered in combination with other antitubercular drugs such as rifampin, pyrazinamide, and ethambutol. This combination therapy helps prevent the development of drug resistance and ensures a higher treatment success rate. The standard regimen for drug-susceptible TB typically lasts for six months, with isoniazid being administered daily or intermittently.
- **Latent Tuberculosis Infection (LTBI)**: Isoniazid is also used for the treatment of latent tuberculosis infection, which is a state where the individual is infected with Mycobacterium tuberculosis but does not exhibit active disease symptoms. The standard regimen for LTBI involves daily administration of isoniazid for 6 to 9 months. This preventive therapy significantly reduces the risk of developing active TB, especially in high-risk populations such as HIV-infected individuals and those with recent TB exposure.

2. RIFAMPIN

- **Active Tuberculosis**: Rifampin is utilized for the treatment of all types of TB. The initial phase of short-course therapy for TB typically involves a 3-drug regimen of rifampin, isoniazid, and pyrazinamide administered over approximately 2 months.
- **Latent Tuberculosis Infection (LTBI)**: Rifampin-based regimens have recently replaced isoniazid as the primary recommendation for treating LTBI. Rifampin-based regimens have demonstrated similar efficacy with a shorter treatment course and better completion rates.

3. ETHAMBUTOL

- **Active Tuberculosis**: Ethambutol is used in combination with other antituberculosis medications such as isoniazid, rifampin, and pyrazinamide. It is particularly important in areas with high rates of drug-resistant TB to prevent the development of resistance.
- **Drug-Resistant Tuberculosis**: Ethambutol is included in the treatment regimen for patients with suspected or confirmed drug-resistant TB, especially when resistance to isoniazid or rifampin is suspected.

4. PYRAZINAMIDE

- **Active Tuberculosis**: Pyrazinamide is a critical frontline TB drug that plays a unique role in shortening the treatment period from 9–12 months to 6 months. The inclusion of pyrazinamide with isoniazid and rifampin forms the basis for current short-course chemotherapy.
- **Mechanism of Action**: Pyrazinamide kills a population of M. tuberculosis persisters that are not killed by other drugs, contributing to the sterilizing activity of the regimen.

5. STREPTOMYCIN

- **Active Tuberculosis**: Streptomycin is used when an injectable drug is needed or desirable and in the treatment of infections resistant to other drugs. It is typically administered intramuscularly or intravenously for several weeks, followed by intermittent dosing for several months.
- **Drug-Resistant Tuberculosis**: Streptomycin remains a potentially effective treatment and is important to consider in cases where standard combination therapy may not be tolerated or is otherwise unavailable. It is considered an acceptable alternative therapy among other choice antibiotics with activity against M. tuberculosis, such as rifapentine, rifabutin, linezolid, and certain fluoroquinolone antibiotics.

SCREENING METHODS

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is a life-threatening infectious disease, which was responsible for 1.3 million deaths globally. The treatment regimen for TB mainly comprises antibiotics such as rifampicin (RIF), isoniazid (INH), ethambutol, pyrazinamide, and aminoglycosides. Therapy with these antibiotics must be taken for 6 months to 2 years, which can result in the emergence of drug resistance and poor therapeutic outcomes.

INVITRO METHODS:

- **Microplate Alamar Blue Assay**

Mycobacterium tuberculosis H37Ra was cultured to mid-log phase, with an optical density (OD) of 0.6 (~5 × 107 colony-forming units [CFU]/ml) at 600 nm. One hundred microliters (5 × 104 CFUs) of bacterial suspension were added to each well of 96-well microplates. Then, 10 μM of the compound was added to each well. DMSO was used as the negative control, and rifampicin (RIF) was used as the positive control. The plates were sealed and incubated at 37°C for 6 days. A 10% (v/v) solution of Alamar Blue was then added to each well. The anti-Mtb effect of each compound was determined based on the color change. The compound was considered to be inactive against Mtb if the color changed from blue to red.

RESULT

The large-scale screening of the compounds in microplates showed that antibiotics including orbifloxacin, prulifloxacin, nadifloxacin, disulfiram, and mithramycin, and antimicrobial peptides exhibited significant anti-Mtb activity. Amongst the compounds tested, the fluoroquinolones (orbifloxacin, prulifloxacin, and nadifloxacin) and mithramycin were found to show the best anti-TB activity.

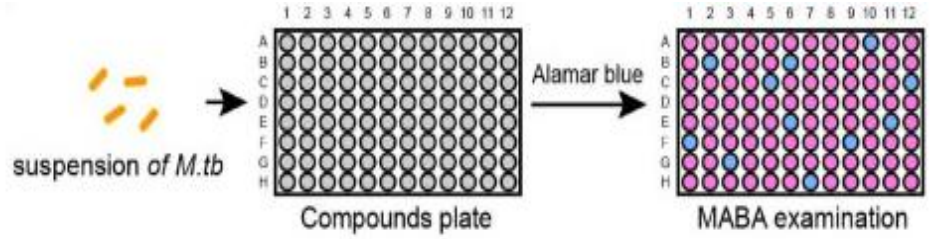


Fig.16 Microplate Alamar Blue Assay

- **Cell Line Culture and Cytotoxic Assays**

5 × 103 RAW264.7 or THP-1 cells were seeded into 96-well microplates and incubated overnight. The cells were treated with a range of concentrations of different drugs or peptides for 48 h, and it also applied to the 50% effective cytotoxic concentration value determination. Then, 10 µl of the MTS kit reagent solution was added. DMSO was used as the negative control. After 4 h of incubation, cell viability and cytotoxicity were measured using a CellTiter 96 Aqueous One Solution Cell Proliferation Assay and the absorbance was recorded at 490 nm using a 96-well microplate reader.All cell experiments were conducted using no more than four generations of subculture after thawing of the cell stocks. A mycoplasma-free test was performed by using the myco-blue mycoplasma detector.

RESULT

The cytotoxicity of the selected antibiotics and peptides was tested using the MTS method. Orbifloxacin also displayed a very high safety cytotoxic concentration 8 µg/ml to Raw264.7 cell with but showed distinct cytotoxicity toward THP-1 cells even at low concentration. The peptides did not show any cytotoxicity up to 64 µg/ml; the maximum safe concentration was found to be 16 µg/ml.

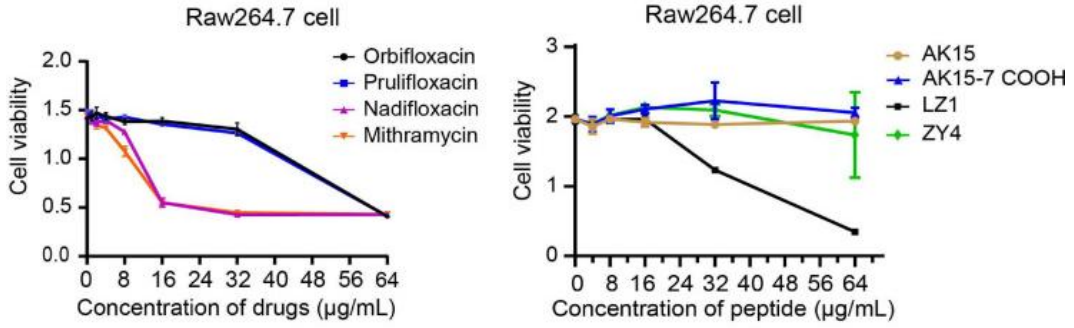


Fig.17 Cell Line Culture and Cytotoxic Assay

- **Determination of the Minimum Inhibitory Concentration of Selected Compounds.**

  For minimum inhibitory concentration (MIC) analysis, 100 µl of the bacterial suspension was prepared as above and added to the wells of a 96-well plate. Ten microliters of twofold serial dilutions of screened drugs (from 0.012 to 12.8 µg/ml) and peptides (from 1 to 512 µg/ml) were added to the indicated well. Bacterial growth was assessed visually after culture for 1 week.

- **Checkerboard Test of Antimicrobial Orbifloxacin Combinations**

  Checkerboard assays with Mtb H37Ra were used to assess the synergic activity of orbifloxacin in combination with first-line anti-TB drugs. The activity of orbifloxacin was tested in combination with RIF, INH, streptomycin, kanamycin, ethambutol, and ethionamide. A total of 100 µl of the bacterial suspension was added to 96-well microplates as previously described. The plates were incubated at 37°C for 7 days, and bacterial growth was visually assessed.

- **Drug Susceptibility Testing Against Intracellular Mtb**
- Assessment of Drug Susceptibility for Intracellular Mycobacterium tuberculosis

  For in vitro antimicrobial activity evaluations, 2 × 10^5 RAW264.7 cells were grown in 12-well plates overnight and subsequently infected with H37Ra at a multiplicity of infection of 5 for 4 hours at 37°C in a 5% CO2 atmosphere. Cells were washed three times with sterile PBS to terminate the infection. Each well received fresh DMEM supplemented with 10% FBS, orbifloxacin (4 µg/ml), peptide (12.8 µg/ml), and a combination of both orbifloxacin and peptide. Kanamycin acted as a positive control against extracellular Mycobacterium tuberculosis. After a 48-hour incubation, the cells were washed with PBS and then lysed with sterile 0.1% Tween 80. Cell lysates were diluted and then injected onto 7H11 agar plates. Colony-forming units were enumerated after an incubation duration of 3 to 4 weeks.

- **Whole-Genome Sequencing of the Drug-Resistant Mutants**

Drug resistance in Mtb H37Ra was induced by co-culturing with orbifloxacin at concentrations from 1/4-fold to 10-fold MIC over three generations, culminating in the final generation demonstrating notable growth of Mtb in the presence of 10-fold MIC of orbifloxacin. Drug-resistant mutants were isolated from a single colony and cultured on a 7H11 agar plate enriched with a tenfold MIC concentration of orbifloxacin. Genomic DNA was extracted using the cetyltrimethylammonium bromide–phenol chloroform method. Bacterial pellets were re-suspended in GTE lysis buffer (50 mM glucose, 25 mM Tris, 10 mM EDTA; pH 8.0) with lysozyme at a final concentration of 100 µg/ml and incubated at 37°C overnight. RNase A (10 µg/ml), a 2% SDS solution, and protease K (10 µg/ml) were combined, and the resulting mixture was incubated at 56°C for 2 hours. Chloroform–isopentanol and 75% ethanol were utilized to isolate and purify the genomic DNA. The isolated DNA was quantified using a Nanodrop 2000 spectrophotometer. The DNA (2 µg) was fragmented using sonication and subsequently purified with a gel extraction kit. Fragmented DNA was repaired using the Hieff NGS Fast-Pace End Repair and subsequently ligated with a Y-adapter. Paired-end DNA libraries were produced using a 14-cycle PCR amplification for Illumina next-generation sequencing.

INVIVO METHODS :

- **CYTOKINE ASSAY**

  Cytokine Assay: Six female mice, aged 6 to 8 weeks and weighing around 20 ± 2 grams, were housed under specific pathogen-free conditions in the laboratory. All animals were randomly assigned to three groups, each including 6 to 8 mice. Mice were intravenously administered 0.2 ml of Mtb H37Ra suspension at a concentration of 5 × 10^6 CFU/ml over a period of 4 days. The infected mice were subsequently administered orbifloxacin orally at a dosage of 50 mg/kg daily. Isoniazid acted as a positive control at a dosage of 12.5 mg/kg, whereas PBS served as a negative control. The drugs were administered to the mice for 7 days post-infection. The mice were euthanized 18 days after infection. RNA was extracted from the lung tissue of mice using the chloroform–isopropanol extraction method. The lungs were employed for histopathological analysis and colony-forming unit assessment.

RESULT

The mice in the orbifloxacin and isoniazid (INH) groups demonstrated slight weight loss and a notable reduction in CFUs relative to the PBS control group. No significant difference was noted between the groups receiving orbifloxacin and INH. Histopathological examination of lung tissues revealed that PBS-treated mice had much higher infiltration of inflammatory cells, such as macrophages and lymphocytes, in contrast to drug-treated mice. In PBS-treated mice, thickening of the alveolar wall, narrowing of the alveolar space, and accumulation of erythrocytes within the pulmonary cavity were noted. In contrast, the lesions were markedly less severe, and a diminished quantity of macrophages was noted in the alveolar cavity of the mice treated with orbifloxacin. We additionally analyzed cytokine expression levels in the pulmonary tissues of the subjects. The levels of IL-6 and IL-1β were significantly decreased in the orbifloxacin-treated group relative to the PBS-treated group. No notable disparities were seen in the expression of TNF-α among the groups. Our data suggest that orbifloxacin exhibits considerable in vivo anti-Mtb activity and may be considered a potential therapeutic drug for tuberculosis treatment.

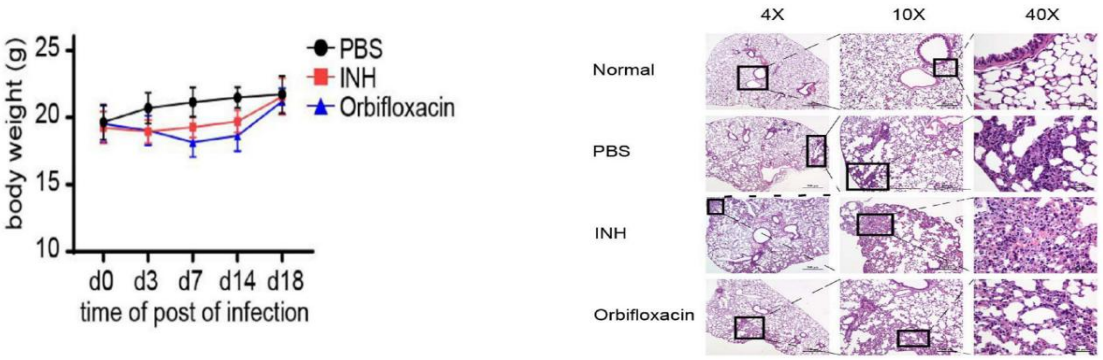


Fig.18 Cytokine Assay

Summary:

This study evaluates the effectiveness of isoniazid (INH) in treating tuberculosis (TB) compared to other first-line anti-TB drugs, such as rifampicin, ethambutol, and pyrazinamide. Isoniazid, a potent bactericidal agent, is an essential element of tuberculosis treatment due to its efficacy against actively dividing Mycobacterium tuberculosis. The study investigates therapeutic results, patterns of medication resistance, side effects, and patient adherence. Research indicates that isoniazid, when used in conjunction with other first-line drugs, has significant bactericidal effectiveness and shortens treatment duration. Resistance to isoniazid is concerning; nonetheless, combination therapy mitigates treatment failure . Isoniazid exhibits comparable efficacy to

rifampicin, however with less adverse effects. Ethambutol and pyrazinamide are primarily utilized to prevent resistance and target certain bacterial populations.

Conclusion:

In conclusion, isoniazid is an essential and effective first-line medication for tuberculosis treatment, particularly when combined with other medicines. Its potent bactericidal effectiveness and relatively favorable side effect profile make it indispensable. Nevertheless, the rise of isoniazid resistance necessitates stringent monitoring and adherence to treatment protocols. The role of isoniazid in tuberculosis treatment is essential; however, optimal care requires a multi-drug regimen to prevent resistance and improve patient outcomes.

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