Anti-mycobacterial agents

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**Introduction**

- Mycobacteria is a genus of slow growing, acid-fast bacilli belonging to the Mycobacteriaceae, which include the organisms responsible for tuberculosis and leprosy as well as other, less common diseases.
- Mycobacterium can be stained by basic stain such as Gram stain (usually staining is difficult and increased with the aid of heat), however, they resist de-staining by acidic alcohol (i.e. acid-fast).
- Mycobacterium has abnormally high lipid content in the cell wall which is responsible for its difficult staining. This cell envelope is unique in structure and complexity and is responsible for:
  - High Pathogenicity or virulence
  - Multiple drug resistance
  - Low cell permeability
  - Low immunoreactivity and inhibition of antigen responsiveness
  - Disease persistence and recrudescence.
Mycobacterium tuberculosis

- Mycobacterium tuberculosis is the principal causative agent of TB in human.
- It is a slow growing Gram-positive rod-shaped bacterium that has a thick, rigid, and hydrophobic cell wall which serves to protect the organism from the environment, making it highly impermeable to conventional antimicrobial agents.
Antimycobacteria agents

• The first breakthrough in antitubercular chemotherapy was in 1938 by the use of sulfanilamide which has weak bacteriostatic properties.
• Sulfone derivative dapsone (4,4’-diaminodiphenylsulfone) was used later, however high doses are required which are too toxic.
• Aminoglycoside antibiotic streptomycin was used later in 1944.
• Para-amino salicylic acid (PAS) was then used and then isoniazid (INH), ethambutol and the semisynthetic antibiotic rifampin.
Combination therapy, with the use of two or more antitubercular drugs was used to reduce emergence of resistant strains of *M. tuberculosis*.

The choice of individual agents in combination depends on:

1. Location of disease (pulmonary, urogenital GIT, neural)
2. Susceptibility tests
3. Pattern of resistance
4. Physical conditions and age of patient
5. Toxicities of each agent

(INH + ethambutol ± streptomycin) was preferred but toxic due to streptomycin. So the new combination include:

- Rifampin instead of streptomycin
- Pyrazinamide (good sterilizer) instead of ethambutol

The treatment regime is for 6 months to 2 years
TB is treated in two phases. There is an initial phase and a continuation phase and depending upon the patients ability to comply with the drug regime:

1. Phase-I: Here there is the concurrent use of at least three drugs to reduce the bacterial population as rapidly as possible in order to prevent resistance.
   - as a combination preparation or “triple therapy”.
   - Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA).
   - Streptomycin (SM) may be used in cases where resistance to INH has been established.
   - The initial phase drugs are normally used for two months

2. Phase-II: After the initial phase, a further four months of chemotherapy is carried out using preferably a combination of RIF and INH.
Potential targets in *M. tuberculosis*

- Targeted pathways should be unique to the *Mycobacterium*.
- Many agents have been introduced to target certain metabolic sites:
  1. Inhibit cell wall biosynthesis.
  2. Affect protein biosynthesis.
  3. Affect DNA replication and transcription.
  4. Inhibit fatty acid synthesis (FAS).
• MIC measured as µg/ml

Incorporation of FA into cell wall

Similar to sulfonamides “competes PABA”

Unknown

Cell wall synthesis

Inhibit DNA replication

MIC measured as µg/ml

Fatty acid synthesis

• MIC measured as µg/ml

Incorporation of FA into cell wall

Similar to sulfonamides “competes PABA”

Unknown

Cell wall synthesis

Inhibit DNA replication
Mycobacterial cell envelope contains:

1. Interior plasma membrane
2. Peptidoglycan layer that give mechanical support to the cell and composed from N-glycoyl-muramic acid (NAM) linked to N-acetylglucosamine (NAG) which in turn linked to
3. Polysaccharide layer: which composed of polygalactan which in turn linked to arabinan which in turn linked to
4. Mycolic acid residues that are spanned with number of free nonpolar and polar lipids (the phthiocerol lipids and the glycopeptidolipids, respectively
• It is the mycolic acid layer that provides the waxy envelope for mycobacterium cell wall

• Mycolic acids are β-hydroxy C\textsubscript{54-63} fatty acids with a long α-alkyl side chain of C\textsubscript{22-24} in length.

• It plays important roles in the mycobacterium, including resistance to chemical injury; resistance to dehydration, low permeability to polar molecules and allow the bacterium to grow readily inside macrophages

• α-mycolic acids are the predominant form (70%)

• The enzyme inhA, produced under the control of the \textit{inhA} gene, is an NADH-dependent, enoyl reductase protein thought to be involved in double-bond reduction during fatty acid elongation
INH cause lose of lipid envelope by inhibition of mycolic acid synthesis by inhibition of NADH-dependent reductase of 2-trans-enolyacyl carrier protein (which is required for fatty acid elongation)
Antimycobacterium agents: Isoniazide

- **Isoniazid**
  - Isonicotinic acid hydrazide, isonicotinyl hydrazide, or INH. Is white crystalline and very soluble in water
  - INH is very effective against intracellular and extracellular bacilli, however, resistant strains of M. tuberculosis emerged, although partially overcomed with combination therapy
  - It is bactericidal against replicating organisms, but be only bacteriostatic on semi-dormant and dormant organisms
  - **Synthesis:** INH is prepared by reacting the methyl ester of isonicotinic acid with hydrazine (NH$_2$NH$_2$)
Isoniazide (Cont.)

• INH is active on growing bacilli and not on resting forms.
• INH is a prodrug and need to be activated by catalase-peroxidase enzyme (KatG).

• Resistance to INH is developed by:
  1. Mutations in NADH-dependent reductase
  2. Absence of KatG gene in mycobacterium

Note: Ethionamide is a prodrug similar to INH, however is being activated by other enzyme than KatG. Therefore, resistance to ethionamide can be acquired by mutation of NADH-dependent reductase but not by absence of KatG
INH is a prodrug activated by enzyme KatG (exhibit catalase-peroxidase activity) to reactive species capable of acylation other molecules. Activated INH is an electrophile species that acylates the four position of the NADH. The acylated NAD is no longer capable of catalyzing the reduction of unsaturated fatty acids, which are essential for the synthesis of the mycolic acids.
• Catalysis of reduction-oxidation reactions by nicotinamide adenine dinucleotide

\[
\text{Rib} \overset{\text{ADP}}{\rightarrow} \overset{\text{Reduction}}{\text{NAD}^+ + \text{H}^+ + 2\text{e}^-} \overset{\text{Oxidation}}{\rightarrow} \overset{\text{NADH}}{\text{Rib} \overset{\text{ADP}}{\rightarrow}}
\]
FIGURE 36.2 Reaction products formed from catalase-peroxidase reaction with isoniazid (INH).
Isonicotinic acyl-NAD complex will tightly bound to the active site of InhA thus preventing access of the cofactor of NADH as well as the substrate of enoyl-AcpM substrate.
Substitution on the $N$-2 position resulted in active compounds ($R_1$ and/or $R_2 = \text{alkyl}; R_3 = \text{H}$).

Whereas any substitution of the $N$-1 hydrogen with alkyl groups destroyed the activity ($R_1$ and $R_2 = \text{H}; R_3 = \text{alkyl}$).

None of these changes produced compounds with activity superior to that of INH.

Isoniazid hydrazones derivatives are active but are unstable in GIT and releases the INH, therefore, the activity is due to INH.

Isonicotinic acid hydrazides

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Metabolism of INH

- Isoniazid is extensively metabolized to inactive metabolites. The major metabolite is \( N \)-acetyl isoniazid. The enzyme responsible for acetylation is cytosolic \( N \)-acetyltransferase.

- Other metabolites include isonicotinic acid, which is found in the urine as a glycine conjugate and hydrazine. Isonicotinic acid also may result from hydrolysis of \( N \)-acetyl isoniazid, but in this case, the second product of hydrolysis is acetyl hydrazine.

- Acetyl hydrazine is acetylated by \( N \)-acetyl transferase to inactive diacetyl product.

- It has been suggested that a hydroxylamine intermediate is formed that results in an active acetylation agent.
ISONICOTINYL HYDRAZONES OF PYRUVIC ACID

ISONICOTINYL HYDRAZONES OF α-KETOGLUTARIC ACID

ISONICOTINIC ACID + HYDRAZINE

N-ACETYLISONIAZID

HEPATOTOXICITY

ACETYLHYDRAZONE

DIACETYLHYDRAZONE

FIGURE 36.6 Metabolism of isoniazid.
• *N*-Acetylhydrazine has been postulated to serve as a substrate for microsomal P450, resulting in the formation of a reactive *N*-hydroxylamine intermediate that forms acetyl radical/cation which is capable of acetylation of liver proteins, in turn resulting in liver necrosis.

**FIGURE 36.7**  Acylating metabolite of isoniazid.

- Acetylation of liver proteins
- Hepatotoxicity
Toxicities of INH

• Peripheral neuritis: due to antagonism of the coenzyme of pyridoxal phosphate. Neuritis can be reduced by co-administration of pyridoxine.
• Sever hepatoxicity: if combined with rifampin
• GI disturbances
• **Ethionamide**

• 2-Ethylthioisonicotinamide occurs as a yellow crystalline material that is sparingly soluble in water.

• It has weak bacteriostatic in vitro but being bactericidal in vivo due to its lipid solubility.

• 2-substitution enhances activity in thioisonicotinamide series but not in isonicotinamide

• Ethionamide has good oral bioavailability and wide distribution in the body.

• It is used for INH resistant strains
• **Pyrazinamide**

• Pyrazinecarboxamide (PZA) occurs as a white crystalline powder that is sparingly soluble in water and slightly soluble in polar organic solvents.

• It is isosteric heterocyclic analogs of nicotinic acid.

• is not active against dormant bacilli, thus cannot be used for long-term therapy

• Pyrazinamide is maximally effective in the low pH environment that exists in macrophages (monocytes).

• It may be bioactivated to pyrazinoic acid by an amidase present in mycobacteria. Mutation of pyrazinamidase → resistance

• Mechanism of action is unknown and not like isoniazid and ethionamide (i.e. not involve mycolic acid synthesis)
The metabolism of pyrazinamide include hydrolysis by hepatic microsomal pyrazinamidase into pyrazinoic acid, which may be then, oxidized by xanthine oxidase to 5-hydroxy pyrazinoic acid. The later compound may appear free either in the urine or as a conjugate with glycine.
• **Ethambutol**
• Ethambutol, (+)-2,2'-(ethylenediimino)-di-1-butanol dihydrochloride, or EMB, is a white crystalline powder freely soluble in water and slightly soluble in alcohol.
• Ethambutol is active only against dividing mycobacteria and not on dormant (encapsulated) bacteria
• It inhibit the incorporation of mycolic acids into the cell wall
• It is stereospecific (has chiral center with *dextro* isomer is 16x more active than *levo*)
• **Cycloserine**
  
  • D-(+)-4-Amino-3-isoxazolidinone is an antibiotic isolated from Streptomyces species
  
  • D-cycloserine is stereochemically related to D-serine.
  
  • Antimicrobial activities of D-cycloserine and L-cycloserine are almost similar
  
  • D-cycloserine inhibit two key enzymes for cell wall synthesis, namely: D-alanine racemase and D-alanine ligase.
  
  • D-cycloserine competes L-alanine for alanine racemase (a pyridoxal phosphate-dependent enzyme that convert L-alanine to D-alanine)
  
  • D-cycloserine is a rigid analog of d-alanine, thus having better affinity due to entropy gain
  
  • D-cycloserine produce CNS side effects
  
  • Resistance is associated with an overexpression of D-alanine racemase
FIGURE 36.15  Sites of action of $d$-cycloserine: 1, $d$-alanine racemase; 2, $d$-alanine ligase.
• **Para-aminosalicylic** acid is also used as anti-mycobacterium

• It’s solubility is slight in water, but can be improved by converting it to metallic salts.

• It is polar and distribute into most body fluids with the exception of CSF (?). It is excreted primarily in the urine as unchanged and metabolized (N-acetylated)

• PAS has similar structure to P-amino benzoic acid (PABA) thus it though to prevent folic acid synthesis (similar to sulfonamides)
Rifamycins are a group of chemically related antibiotics obtained by fermentation from cultures of *Streptomyces mediterranei*.

Rifamycins have macrocyclic ring bridged across two nonadjacent positions of an aromatic nucleus.

Several semisynthetic analogues of rifamycins are available which are active against Gram-positive bacteria (including *M. tuberculosis*), Gram-negative bacteria as well as some viruses.

Inhibitors of DNA directed RNA polymerase of bacteria but not of human. In viruses, it inhibit specific polypeptide conversion that is involved in formation of virus particle.

Rifampicin is used as anti-tuberculosis.

Rifampicin causes hepatotoxic effects especially if combined with isoniazid.