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ROLE OF MULTIDRUG TREATMENT IN MYCOBACTERIUM TUBERCULOSIS: AN OVERVIEW

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Abstract: **Tuberculosis is a bacterial infection, affecting the lungs. Tuberculosis has been a leading cause of death since many years. With the evolution of science and technology, many related drugs have been discovered. They have a prescribed schedule and the dosage relies on symptomatic treatment. This is because with time and doses, the microorganism evolves and adapts new methods to resist antibiotics. This causes multi-drug resistance in organisms, leading to an increase in the potential of the bacterial colonies. This poses a threat to the person and so on. This paper highlights the development of anti-tuberculin drugs, their role in treatment and their mechanisms of resistance.**

Keywords: Mycobacterium tuberculosis**,** vir genes, peptidoglycan, potency, antibiotics Chemotherapy, Promin, Dapsone, Streptomycin, PAS, isoniazid, pyrazinamide, ethambutol and rifampicin, antibiotic resistance crisis, HGT.

I. INTRODUCTION

Tuberculosis can be categorised an air-borne disease that kills approximately two million people each year. It is a much serious disease compared to other infectious diseases like plaque and cholera. The occurrence of Mycobacterium genus of bacteria, just like other actinomycetes were found initially in the soil and gradually some species evolved to live in mammals. This is how M. tuberculosis was discovered.

Tuberculosis has been a challenging disease since ages because of its competitive and modifying nature. The organism contains genes that code for proteins potentially involved in virulence, vir genes. Virulence can be defined as the extremity a disease or a pathogen can cause. Many such proteins have been discovered and sequenced. This has been remarkably utilised to formulate drugs, vaccines and more selective diagnostic reagents. But none of them have been successful to eradicate or treat the disease completely. Well, many of these genes and their encoded proteins as bacterial targets are yet to be discovered. These mechanisms make it highly infectious, and hence multidrug resistant.

Because of the multiple proteins encoded, the variation in structure and its rapid adaptability, the *Mycobacterium* requires multiple chemotherapies to be treated successfully. Inadequate treatment of patients leads to emergence and spread of the multi-drug resistant Mycobacterium tuberculosis strains. It is thus, the combinatorial drug principle used to maintain a constant drug level in the blood making it effective in a long run.

II. OBJECTIVE

M. tuberculosis, which is a GPB has an exceptionally thick cell wall containing multiple layers. The wall is impermeable, which acts as an effective barrier for the penetration of antibiotics. The hydrophobic layer has an inter-layer space and therefore acts similar to GNB cell wall. Peptidoglycan is covered by arabinogalactan layer. The outermost layer has mycolic acids in the form of long-chain fatty acids that form a waxy, non-fluid barrier preventing penetration of hydrophobic and hydrophilic molecules. They can be seen by acid fast stain. Fig. 1 represents microscopic view of M. tuberculosis.

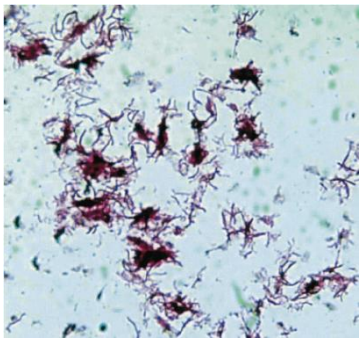

Fig 1: Acid fast stained Mycobacterium tuberculosis

Discovery of novel antibiotics that can harm the bacterial cell, avoiding growth of them has been a challenge for researchers.

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a. Discovery of Tuberculosis:

In 1882, M. tuberculosis was discovered by Robert Koch. This was the first instance in discovering the causative organism, and thus creating a quotient for learning more about it. The mortality rates that occurred in the era of Industrial revolution were sufficiently high, although it declined in a couple of years. The treatment or care given to such patients with all forms of tuberculosis was in the sanatorium. The other major treatments used in critical cases involved artificial pneumothorax, artificial pneumoperitoneum, thoracoplasty, plombage, phrenic nerve crush, and lung resection, since lung is the main organ affected. By the time postoperative care and surgeries became sufficiently advanced, chemotherapy came into play.

Chemotherapy in ancient terms was defined as a magical chemical bullet that had the capability of killing microscopic particles without affecting the human host. It was a chemical used therapeutically to combat the effects of a particular disease such that its potency is high enough to damage the microscopic strains only. A very important characteristic of a therapeutic agent is that it will not produce any side effects and will not lead to relapse of a disease ever. The history of development of drugs is represented in Fig. 2.

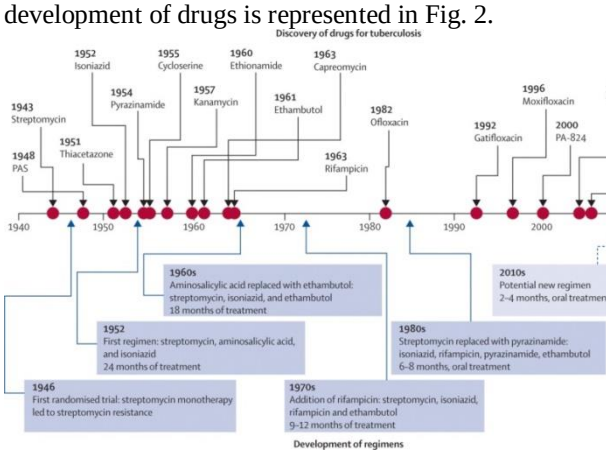

Fig 2: Events that led to the development and gradual progress in the discovery of drugs for Tuberculosis

b. Benefits of antibiotics:

Antibiotics have increased the lifespan of patients and given them a better quality of life, even after dealing with tuberculosis. Drug-resistant M. tuberculosis strains or antibiotics have also prevented or treated the secondary infections present in patients that receive chemotherapies or are sufferers of AIDS. This is done to reduce the chances of antibiotic resistance within the community. It also serves as an advantage to patients who have undergone surgeries such as transplants in joints, heart or other organs. Chronic diseases such as diabetes and arthritis have increased with time. In such cases, development of multipurpose antibiotics serves as a boon.

In some places where sanitation is poor, antibiotics decrease morbidity and mortality caused by infections.

c. Development of antibiotics:

The role of an antibiotic treatment is to reduce the bacterial load in the lungs. The clinical and public health management of the patients and their contacts is complicated, yet crucial. Public health measures such as isolation and cough etiquette along with the accurate dosage is useful to reduce the chances of transmission. A lot of development has been taken place in the last couple of decades.

Antibiotics such as arsphenamine successfully treated syphilis. This proved to be the first instance of success of a chemotherapeutic agent. Parallel to it, penicillin and sulfonamido-chrysoidine were synthesized. But none of them could be effective against tuberculosis. This led to the discovery of a chemotherapeutic agent that would be appropriate for tuberculosis.

A drug named Promin which was a derivative of Dapsone, discovered by Henshaw and Feldman was the first composition believed to be efficient against tuberculosis but it failed. Sulphonamides were also tested in guinea pigs and humans for trials. Guinea pigs treated with Streptomycin-isolated from *Streptomyces Griseus* showed significant improvement. Schatz and Waksman in the year 1944 stated that the drug could be prescribed for the treatment as it was bactericidal. The duration of the treatment as proposed by the United Kingdom Medical Research Council Tuberculosis Unit was 6 months.

By that time roughly 1945, experiments in laboratories and patients performed by Lehman had observable improvements in their conditions. The novel magical synthetic compound was PAS (para amino-salicylic acid). A notable issue here was that although PAS was discovered before streptomycin, both had parallel effects and side effects. Also, if the drug was given one at a time, it produced resistance. Roche, Chemical and Squibb discovered isoniazid most immediately. Dedicated studies were done towards the chemistry, pharmacology, and effects of isoniazid by American Journal of Respiratory and Critical Care Medicine was done. A conclusion was drawn that it was self-sufficient as it was till then the safest, inexpensive and well tolerated. But with time, the strain was seen to show resistance towards isoniazid. This was the second instance showing that a mono-drug was not enough to combat the bacteria. This gave rise to the principle of triple therapy used-isoniazid, streptomycin with PAS. Till about 15 years, this combination of drugs or combination of drugs was

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considered standard for treatment of *Mycobacterium tuberculosis*.
The drugs that followed after isoniazid are pyrazinamide in 1954, ethambutol in 1961and rifampicin in 1963. It demonstrated the efficacy of a long-term and multidrug therapeutic approach to obtain a bacteriological eradication in pulmonary and extra-pulmonary sites.

d. Overview of essential drugs:

Presently an entire course for tuberculosis is for duration of six months. The course of treatment is represented in Fig 3.

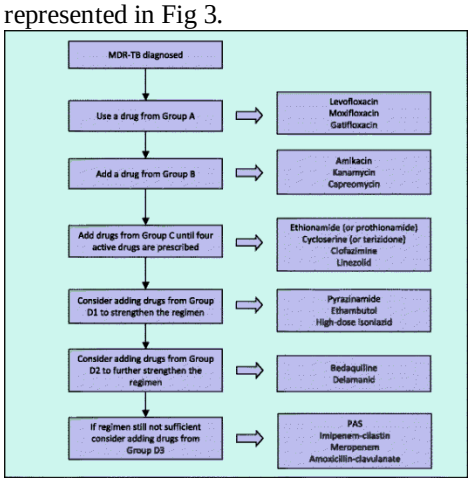

Fig 3: List of the reactivity with drugs

Here, in the first 2 months patients receive three to four drugs, namely rifampin (R), isoniazid (H), pyrazinamide (Z), and ethambutol (E).
In the final 4 months, drugs such as rifampin and isoniazid are continued. Several populations of bacilli strains that keep on mutating and producing resistant strains are present in infected individuals. The strains present in bacteria can be classified as those that grow rapidly, those that grow slowly, and those that multiply sporadically.
The role of rifampin, which is a sterilizing antibiotic, is to gradually reduce bacterial count to zero during the secondary phase of therapy. The biphasic killing of cultures denotes that the rapid multipliers are cleared in the primary growth bactericidal phase, and the slow and sporadic organisms get killed in the later phase of sterilization.
On the other hand, isoniazid and streptomycin (S) have a high bactericidal activity. Slow growing and sporadically multiplying populations of *M. tuberculosis* still persist in the affected organism in spite of antibiotics given to kill them rapidly, the and these may only be cleared by antibiotics possessing “sterilizing” activity.
Pyrazinamide administered during the first two months of treatment along with the other complementary drugs, reduces the overall duration of therapy, without producing any considerable effect when administered alone. Ethambutol is included in the regimen, especially to treat drug-susceptible *M. tuberculosis* that harboured drug resistance. Effective dosing and development is only possible with growth in research and medications, such as rifapentine, rifabutin, and rifalazil, which are derivatives of rifamycin. The list of drugs is presented in Table 1. The side-effects of each drug is represented in Table 2.

e. Antibiotic resistance:

Within a few decades, the cases of tuberculosis with severity have increased at an alarming rate. Novel discovery for treatment of bacterial infection is limited or requires a long time. The other requisite for creating an ideal drug is to update epidemiological data as it not only decides the treatment strategies but also devises ineffective antimicrobial stewardship program in hospitals.
The antibiotic resistance crisis has caused overuse and misuse of these medications and lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements. Conventional antimicrobials or single drugs increase the difficulty to treat relapsable bacterial infections. An adequate combination of effective drugs is essential to reduce the probability of failure, relapse, and selection of resistant strains. The antimicrobial resistance to bacterial pathogenesis has been a worldwide concern as it is associated with high morbidity and mortality rates.
Taking real life issue into context, although the entire course provides effectiveness in treating drug susceptible TB, the lengthy treatments often lead to non-adherence of the patient. The misuse of antibiotics or careless dosage has created selective pressure leading to evolution of M. tuberculosis from mono to multi-drug resistance (MDR), extensive drug resistance (XDR), and eventually total drug resistance (TDR). This catalysed the emergence of M. tuberculosis strains whose resistance to the few available anti-TB drugs kept increasing.
Most infections caused by microorganisms that are resistant to certain antibiotics fail to respond to conventional treatment. This ideally concludes that even the previously treated antibiotics have lost its power.
The bacterial strains contain antibiotic resistance genes (ARGs) that provide protection to them by easily modifying their proteins. Studying about the overall causes of such resistance we get,
Selective evolution of mutant resistance genes over the original sensitive ones leads to a competition between them. If resistance confers an advantage to the bacteria, there is a steady growth of the antibiotic resistant strains. Sequential accumulation of resistance is caused by chromosomal mutations under the selective pressure of antibiotic use. All

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bacteria that are pathogenic, commensal and environmental, mobile genetic elements and bacteriophages, have a reservoir of ARGs- the resistance providing bodies from where the pathogenic bacteria can acquire resistance via mechanisms such as horizontal gene transfer (HGT), transduction and transformation.

The areas of research include developing new generation of antibiotics, combination therapy, natural antibacterial substances and applying nanoparticulate systems. A risk of emerging resistance to antibiotics increased if an exposure to oxidising agent such as hydrogen peroxide is maintained. An exposure to cationic biocide and oxidizing agent, notably to tobramycin and ticarcillin-clavulanic acid resulted in a number of unstable clinical resistances to antibiotics.

Antimicrobial resistance stimulated an increase in research activity, and several promising strategies have been initiated to restore treatments against infections by resistant bacterial pathogens. There has also been a hypothesis where in vitro studies and isolation of drug-resistant M. tuberculosis can lead to sensitisation to a particular antibiotic after repeated exposure to antibiotics. Table 3 represents the list of possible antibiotics.

Drug formulations	Route of delivery	Results	An
INH, RIF, PZA encapsulated PLG nanoparticles	Inhalation; injectable; oral	Increased bioavailability and sustained therapeutic drug levels in the plasma and in the lungs for longer duration than the free drug.	Gu
Nanocrystalline suspensions of clofazimine	Intravenous	Enhanced drug accumulation in liver, spleen and lungs	C5
INH, RIF, PZA loaded alginate nanoparticles	Inhalation	Increased bioavailability; enhanced drug accumulation in liver, spleen and lungs above minimum inhibitory concentration	Mi
INH and Rifabutin loaded PLA microparticles	Inhalation	Increased bioavailability and improved pharmacological index.	BA
INH and RIF loaded PLA microparticles	Inhalation	Enhanced nitric oxide and TNF-α production by infected macrophages.	Sw
RIF-loaded PLGA microspheres	Inhalation	Increased bioavailability and enhanced therapeutic response.	Gu
Liposome-entrapped rifampin microparticle	Intraperitoneal	Increased intracellular uptake of the drug into host peritoneal macrophages and increased the antimicrobial activity against *M. avium.*	Mi
INH, RIF, PZA loaded Alginate microparticle	Oral	Sustained release and increased bioavailability	Gu
INH, RIF, PZA loaded Alginate-chitosan microparticles	Oral	Sustained release and increased bioavailability	Gu
INH and RIF encapsulated liposomes	Intravenous	Increased clearance of *M. tuberculosis* from liver and spleen of infected mice	Mi
Clofazimine encapsulated lipososme	Intravenous	Reduced toxicity and enhanced therapeutic response against *M. tuberculosis* infection.	BA
Ethionamide-loaded PLGA nanoparticles	Oral	Prolonged drug release	Mi

Table 1: List of anti TB formulations, route of administration and model organisms

Drug	Adverse effects
Isoniazid	Skin rash, hepatitis
Rifampicin	Abdominal pain, nausea, vomiting, hepatitis, thrombocytopenic purpura
Pyrazinamide	Arthralgia, hepatitis
Streptomycin	Vestibular and auditory nerve damage, renal damage
Ethambutol	Retrobulbar neuritis, ocular side effects
Thioacetazone	Skin rash, Exfoliative dermatitis
Para-aminosalicylic acid	Anorexia, nausea, vomiting, hypersensitivity reactions
Kanamycin	Vertigo, auditory nerve damage, nephrotoxicity
Ethionamide	Diarrhoea, abdominal pain, hepatotoxicity
Cycloserine	Dizziness, headache, depression, psychosis, convulsions

Table 2: Adverse side effects of anti-tuberculosis drugs

Drug-discovery	Drugs under pre-clinical trials	Drugs under clinical development
Diarylquinolines		Bedaquiline (TMC207)
InhA inhibitors		SQ-109
LeusRS inhibitors	Q201	
Mycobacterial gyrase	SPR-10199	Novel regimens
Inhibitors	SQ609	
Pyrazinamide analogues	CPZEN-45	
Fluroquinolone	DC-159a	Moxifloxacin
Rifamycins		Rifapentine
Nitroimidazole		PA-824

Table 3: Recent development in the anti-tuberculosis drug discovery

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