ANTITUBERCULAR ACTIVITY

The chemotherapy of tuberculosis has been practiced for 2000 years but it has been a clinical reality for last few years. By 1940, series of drugs effective against experimental and then clinical tuberculosis were developed during next 30 years, beginning with dapsone and streptomycin and currently with rifampicin, capreomycin and ethambutol.

Human pulmonary tuberculosis is predominantly caused by Mycobacterium tuberculosis. The infectious character of the disease was established in year 1882 by Koch [1], who isolated tubercle bacillus, which causes tuberculosis. The organisms are rod-shaped, aerobic hard to strain. The organisms are difficult to decolorize even with acidic alcohol.

The pulmonary form accounts for about 90% of tuberculosis. There are tuberculous meningitis, enteritis, laryngitis, osteomyelitis and rapidly fatal form known as miliary tuberculosis or “galloping consumption”. In tuberculosis there is an extensive tissue destruction and live virulent bacilli become isolated in the cavities and debris of necrotic tissue where they are relatively immune to chemotherapeutic attack. Thus a drug will be effective only if it can penetrate the tubercule and the debris and enter the phagocytes within which the parasites are growing and finally penetrate or attack the parasites.

The prevalence of tuberculosis is still very high in many parts of the world and in some countries it is epidemic [2]. Though the antituberculosis drugs are applied since last 25 years, it has not declined as rapidly as anticipated [3]. It has been estimated that out of all infections caused by mycobacterium tuberculosis, 95% of those infected (tuberculin positive) live undisturbed by the presence of tubercule bacilli [4]. The new
cases of active disease largely come from the remaining of 5% minority. World wide this disease is rated as the most dangerous infectious disease in terms of deaths and economic loss [5,6]. World Health Organisation (WHO) authorities estimate that there are about 3 million new cases of tuberculosis each year, adding to a total of at least 20 million cases of tuberculosis with about 3 million deaths from it annually [7]. Even in country like USA with very high standard of living with very good sanitary system and good health service available, it was estimated that each year there are about 48,000 new cases of tuberculosis and about 10,000 deaths due to tuberculosis. In some of the developing countries the infection rate for children under 14 is as high as 70%. These figures show the need of more research work in the field of tuberculosis chemotherapy.

The classical symptoms of tuberculosis do not appear until the phenomenon of hypersensitivity becomes established. The early stages of tuberculosis are symptoms free, and the disease generally goes undetected until the infection is well advanced. When tubercule bacilli enter the lungs they form a focus of infection. Phagocytes ingest them and form a nodule on which calcium phosphate and collagen deposit.

Consideration of the factors which have made tuberculosis one of the most difficult of the bacterial disease to treat chemotherapeutically, has led to the criteria for an effective tuberculostat to be effective in tuberculosis, a drug must have high toxicity for the tissue and organs of the host. It should be able to penetrate the barriers interposed between the cell walls of macrophage, epitheloid and giant cells. Finally, because the drug would have to be used for a long time, it should be slow in producing resistant strains.
Chapter IV: ANTIMICROBIAL ACTIVITY

Calmetta [8] developed a strain of Mycobacterium Tuberculosis bovin, known as Bacillus of Calmetta and Guerin. It has been administered to millions of humans in the hope of producing prophylaxis. Recent progress in chemotherapy of tuberculosis is due in large measure to devising successful, practicable animal testing methods. In the last 40 years a number of effective drugs have been made available, because of these modern testing methods.

In the search of few antimicrobial agents, demonstration of in vitro activity against the virulent strain of Mycobacterium tuberculosis $H_{37}R_v$ is one of the simplest preliminary tests.

Systematic and scientific work on antituberculosis compounds were started from 1940 when Ristet et al [9-10] in France and Feldmann et al [11] in U.S.A. discovered that diamino diphenyl sulphone (Dapsone) and its glucose-bi-sulphite derivatives were having distinct effect on tuberculosis. Limited success of this compound led scientists to the screening of other derivatives of diamino diphenyl sulphone but with little success.

PAS has been widely accepted as a clinically useful tuberculostat. Though the activity of his compound is very moderate, it is more acceptable because of its low toxicity even at high dosage level.

Isoniazid is one of the most effective antituberculous drugs but when administered alone, a quick emergence of resistant strains follow, so it should be combined with other antituberculous drugs. INH is superior to other tuberculostats, since it can inhibit intracellular tubercule bacilli, in the same concentration as in the test tube [12-13].
Kushner and his coworkers [14] introduced pyrazinamide as an antituberculosis drug in 1952. Its activity was greater than that of PAS, Cycloserin, Viomycin but less than INH or Streptomycin. Extensive research on N,N-diisopropyl ethylene diamine led to one of the most potent and currently widely used antitubercular compound ethambutol [15].

Tuberculosis strains are now emerging that are resistant to combinations of all first line drugs and most second line treatment drugs that are held in reserve for treating TB that shows resistance to first line regiments [16]. The third factor is that new antitubercular drug developments have been neglected during the past 30 years. These observations are frightening when linked to the present HIV/AIDS epidemics. It is believed that HIV can trigger the reactivation of latent TB, which can then accelerate the one set of AIDS in patients with HIV active TB and is one of the commonest indicators of HIV infection.

In view of the epidemic levels of both active and latent forms of this disease, it is essential to accelerate the development of new more effective drugs. This will require a better understanding of how current drugs work in vivo and in vitro. In current therapeutic use, the drug is administered at daily dose of 15-25 mg/kg. in combination with other antituberculous agents, to prevent emergence of resistance strains [17].

With the discovery of Nalidixic acid in urine quinoline family of antimicrobials has attained great significance [18]. Numerous intracellular bacteria including Chlamydia, Mycoplasma, Legionella and Brucella species as well as Mycobacterium tuberculosis are inhibited by achievable Ciprofloxacin concentration. It has a quinoline nucleus in which substitution at position 1,3,4 appears essential for antibacterial
activity, likewise substitution at position 6, and 8 appears primarily responsible for increased antibacterial activity [19].

Formazons are known to be useful agent in various diseases like viral and bacterial infection and Parkinson’s diseases. A number of formazons have been tested and reported as antitubercular agents [20-25]. Realising the medicinal importance of azo compounds, quinoline derivatives and sulfonamides it was considered worthwhile to incorporate these moieties and to study their antitubercular activity.

In the present work selected compounds synthesized in chapter – II of this thesis were tested for their antitubercular activity against INH sensitive strains at 12.5µg/ml.
EXPERIMENTAL

Materials and Methods:

The tubercular property of the compounds was tested by different methods. The two methods most commonly used for susceptibility testing of M. tuberculosis are the method of proportion and the broth radiometric methods. The method of proportion is an agar-based method in which the number of colonies on the control medium is compared to the number of colonies on drug-containing media. Because growth of colonies is necessary for interpretation, this method requires three weeks of incubation. Although the radiometric broth method is much more rapid, the method is semi-automated, and generates radioactive waste.

BacT/ALERT 3D Detection System:

Principle:

The MB/BacT Detection System and the BacT/ALERT 3D employ a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide dissolved in the culture medium. Carbon dioxide is produced as the organisms metabolize the substrates in medium. When growth of the microorganisms produces CO₂, the color of the sensor at the bottom of each culture bottle changes from dark green to lighter green or yellow. The lighter colour results in an increase in reflectance units as monitored by the system. Bottle reflectance is monitored and recorded by the instrument every ten minutes.

The rate and amount of CO₂ production is indicative of the rate and amount of growth of the organism in the presence of an antibiotic, compared to the rate of growth in a
drug free proportional control, determines the susceptibility profile of the microorganism.

The BacT/ALERT 3D System is based on principles similar to those employed by the agar proportion and broth radiometric methods. An organism is determined susceptible when the antibiotic-containing bottle is not detected positive, or has a positive time to detection that is greater than that of the direct control, and greater than that of 1:100 proportional control. An organism is determined resistant when the antibiotic-containing bottle has a positive time to detection that is equal to, or less than, that of the 1:100 proportional control.

Requirements:
1. BacT/ALERT 3D Detection System
2. BacT/ALERT 3D Process Bottles
4. Middlebrook 7H11 or other mycobacterial agar or egg-base medium
5. Sterile Middlebrook 7H9 broth tubes
6. McFarland Standard, 1.0
7. Autoclave
8. Sterile tuberculin syringes with permanently attached needle
9. Alcohol swabs
10. Biological safety cabinet

Preparation of inoculum:
1. Transfer a number of representative colonies from Middlebrook 7H11 or other mycobacterial agar or egg-base
medium into a sterile tube containing glass beads and sterile water. Vortex to disperse clumes. Allow large particles to settle.

2. Transfer the supernatant into another sterile tube and adjust the turbidity to a McFarland No 1.0 using sterile water.

3. Prepare a “seed” bottle intermediate by transferring 0.5 ml of the suspension into a BacT bottle.

4. Wipe bottle tops with gauze that has been soaked in 2% amphyl or any other tuberculocidal agents.

**Preparation of Antitubercular Solution:**

Weigh the 12.5µg/ml of selected compounds and prepare a solution with DMSO and autoclave it for one and half hrs.

**Procedure:**

1. Lable each process bottle to be used in the susceptibility test.

2. Disinfect the rubber septum of each bottle with an alcohol swab or equivalent. Allow the septum to dry before adding either the rehydrated sample compound or restoring fluid to the bottle.

3. Take one bottle and inoculate 0.5 ml inoculum, 0.5 ml sample compound and 0.5 ml restoring fluid.
4. Take another bottle and inoculate 0.5 ml inoculum (1:100 proportional dilution), 0.5 ml restoring fluid and 0.5 ml DMSO and labeled it as standard one.

5. Enter inoculated processed bottle for susceptibility testing into the BacT/ALERT 3D instrument.

**Interpretation of the results:**

**Sensitive:**

The antibiotic-containing bottle is not detected positive, or has a positive time to detection that is greater than that of the direct control and greater than that of the proportional control.

**Resistance:**

The antibiotic-containing bottle has a positive time to detection that is equal to or greater than that of the direct control, but less than or equal to that of the proportional control.
REFERENCES