IN VITRO EFFICACY OF ETHIONAMIDE AND CLARITHROMYCIN IN MYCOBACTERIUM TUBERCULOSIS ISOLATES

Masood Satti*, Shahid Abbasi, Farah Faqir, Shahid Rafi, Tariq Butt***, Karamat Ahmed Karamat******

*KRL Islamabad, AM College Rawalpindi, **Shifa International Islamabad, ***AFIP Rawalpindi, ****Ministry of Health

ABSTRACT

Objective: To determine the sensitivity of clinical isolates of Mycobacterium tuberculosis isolates against ethionamide, and clarithromycin.

Study Design: Cross-sectional study.

Place and Duration of the Study: Department of Microbiology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from June 2003 to June 2004.

Materials and Methods: All routine clinical samples received for acid fast bacilli (AFB) culture and yielding positive growth on Lowenstien Jensen medium and Bactec 460 were included in the study. The isolates were from sputum (n=70), bronchioalveolar lavage (n=10), fine needle aspiration (n=6), lymph nodes (n=7), pleural fluid (n=4), endometrium (n=3). After the identification of M. tuberculosis (MTB) sensitivity was performed against first-line antituberculosis drugs. Then susceptibility of M. tuberculosis isolates against ethionamide and clarithromycin was performed on LJ medium. Mycobacterium H37Rv was used as control strain.

Results: Results were interpreted using resistance ratio method. Out of 100 M. tuberculosis isolates, sensitivity to ethionamide was 93% and 9% to clarithromycin.

Conclusion: Clarithromycin when used alone is ineffective as antituberculosis drug but its efficacy in combination needs to be tested. However ethionamide may be used as an alternative antituberculosis drug.

Keywords: clarithromycin ethionamide, MDR–MTB tuberculosis, susceptibility,

INTRODUCTION

Tuberculosis is one of the oldest diseases known to mankind[1]. Someone in the world is newly infected with Mycobacterium tuberculosis every second. Almost, one-third of the world's population is currently infected with the Mycobacterium tuberculosis. The World Health Organization (WHO) estimates that the largest number of new tuberculosis cases in 2005 occurred in the South-east Asia, which accounted for 34% of incident cases globally. It is estimated that 1.6 million deaths resulted from TB in 2005[2].

Until 60 years ago, there were no medicines to cure tuberculosis. Now, strains that are resistant to a single drug have been documented in every country surveyed and strains of MTB resistant to all major anti-TB drugs have emerged[1]. A particularly dangerous form of drug-resistant MTB is multidrug-resistant TB (MDR-TB), which is defined as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin[3]. The various factors leading to MDR-tuberculosis are deep rooted in our social setup. Addition of HIV and our ignorance will add fuel to the fire[4]. Due to financial constraints drug susceptibility testing is still in rudimentary stages in our setup, however advanced centres in march against tuberculosis have switched to the BACTEC system for quick identification and sensitivity testing. Molecular techniques have also been incorporated in diagnosis and susceptibility testing to save the turnaround time[5].

In this situation of increasing antibiotic resistance to Mycobacterium tuberculosis this study was organized at department of microbiology Armed Forces Institute of Pathology Rawalpindi to find the sensitivity of M. tuberculosis isolated from various clinical specimens against ethionamide, and clarithromycin.

MATERIALS AND METHODS

This study was carried out in the department of microbiology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from June 2003 to June 2004. All routine clinical

Correspondence: Dr Farah Faqir, Department of Microbiology, Army Medical College Rawalpindi
Email: farahfaqir60@gmail.com
Received: 04 Sep 2008; Accepted: 29 Jan 2009

samples received for AFB culture and yielding positive growth of MTB on LJ medium and Bactec 460 TB system were included in the study. The isolates were from sputum (n=70), bronchioalveolar lavage (n=10), fine needle aspiration (n=6), lymph nodes (n=7), pleural fluid (n=4), endometrium (n=3)

Antibiotic susceptibility testing was performed by agar dilution method using Lowenstein-Jensen medium. Stock solutions of first line anti TB antibiotics and ethionamide and clarithromycin were prepared and sterilized by passing through a filter (0.22 µm size). The stock solutions were stored at 40C for up to a month and used as and when required.6

Antibiotics were used in different strengths. The concentrations (µg/ml) used were: Ethionamide: 8, 16, 32, 64, 128, 256µgm/ml. Clarithromycin:- 2, 4, 8, 16, 32, 64 µgm/ml[7].

The antibiotic solution of the required concentration was added to LJ medium and set in to slopes and inspissated at 80^0C. The growth of the Mycobaterium tuberculosis was scraped from fresh LJ slants and suspended in 5ml of distilled water. It was homogenized with glass beads by vortexing. and the tubridity was adjusted to Macfarland scale 1 with distilled water.

Three drops of this suspension were inoculated on the drug containing LJ medium slopes. The bottles were incubated at 37^0C and were aerated twice a week for 03 weeks. Control strain used was H37Rv, which was inoculated on a parallel set of slopes[6].

The M. tuberculosis isolates after identification based on ZN stain, LJ medium and bactec growth characteristics, biochemical tests NAP (Nitro amonopropiophenine) and sensitivity to first line antituberculosis drugs were selected and grouped based on these criteria.

Group-I:- Isolates which were susceptible to all first-line antituberculosis drugs (n=34).

Group-II:- Isolates which were resistant to one or more antituberculosis drug but not both INH and rifampicin (n = 40).

Group-III:- Multidrug resistant (MDR) isolates which were resistant to both INH and rifampicin simultaneously (n= 26).

Method of susceptibility assessment

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that inhibited the growth. Growth was considered to be inhibited if less than 20 colonies appeared on the LJ slope. Minimum inhibitory concentrations (MICs) of the organisms were noted. MICs of the test strains were compared with the control strains inoculated along with the batch tested. Results were evaluated by resistance ratio method. (MIC of test / MIC of control). A ratio of 2 or less was considered as sensitive. Ratio of 4 or greater than 4 was considered as resistant[6].

RESULTS

A total of 100 isolates were included in this study. The sensitivity of to ethionamide in all groups was observed as follows. All of the Group I isolates were sensitive to ethionamide. 27(79%) had a resistance ratio of 1 and 7(21%) had a resistance ratio of 2. In Group II isolates2 (5%) had resistance ratio of 1 and 36(90%) had resistance ratio of 2, thus indicating sensitivity to ethionamide while 2(5%) isolates, resistant to INH had a resistance ratio of 8. In the Group III, (MDR group), 21(81%) isolates had a resistance ratio of 2, while 5(19%) isolates were resistant having a resistance ratio of 8. If we consider all the groups without considering their sensitivity to first line antituberculosis drugs then 93% of the isolates were sensitive to ethionamide(Table).

Clarithromycin sensitivity was found in only 7 (21%) isolates of Group I and 2 (5%) isolates of Group II while rest of the isolates of all the groups showed high MICs resulting in a resistance ratio of 8. Among the 100 isolates sensitivity for clarithromycin was 9 % (Table).

DISCUSSION

In the last decade there has been renewed interest in infections caused by M. tuberculosis. This has been due to resurgence of tuberculosis cases globally. Tuberculosis caused by drug resistant strains of M. tuberculosis is a

Table: Susceptibility of M. tuberculosis against 2nd line anti-TB drugs

Drug	Group I (n=34)	Group II (n=40)	Group III (n=26)	Total (n=100)
Ethionamide	100%	95%	81%	93%
Clarithromycin	21%	5%	0	9%

Key:

- Group I –Isolates of M. tuberculosis sensitive to first line drugs
- Group II- Isolates of M. tuberculosis resistant to one or more drugs
- Group III- Isolates of M. tuberculosis resistant to INH and rifampicin simultaneously

therapeutic challenge for the clinicians. Prevention of MDR-tuberculosis is very important as it is not only beneficial to the patients but also it is cost effective. Tuberculosis and especially MDR-TB is an on going challenge[1,3].

Data for antimicrobial susceptibility testing of second-line drugs is fragmentary in our setup. A comprehensive multi-centre study of susceptibility testing of M. tuberculosis against classical second-line and newer antimicrobial drugs has been carried out using the proportion method with BACTEC 460 system and Middlebrook 7H10 agar by Pfyffer et al[8].

One of the most efficacious of the second-line drugs that is widely used in the treatment of MDRTB is ethionamide. It has long been known that in vivo ethionamide demonstrates more consistent antituberculosis activity than one would expect from in vitro examination of its minimal inhibitory concentration (MIC)[9]. In our study ethionamide was active against all susceptible strains to first line anti-TB drugs of M. tuberculosis. However some INH resistant and MDR-TB strains showed resistance to Ethionamide. Our results correlate with findings of Heym et al who studied 16 strains of M. tuberculosis resistant to both INH and ethionamide[10]. The MICs of ethionamide were > 10µg/ml. Cross resistance between INH and ethionamide has been found to be linked with mutation in inhA locus regulatory region[11]. Clearly there is need for molecular studies to evaluate the intricacies of cross- resistance. Ethionamide shares a common mechanism of action and resistance with INH. In our study susceptibility was performed on LJ medium. This needs a comparative study in both systems. On LJ medium only the susceptibility of M. tuberculosis to a given drug can be interpreted (MIC are usually not done therefore cannot be commented upon). In another study Fattorini et al have reported 4.3% resistance to ethionamide among INH resistant isolates. In the present study M. tuberculosis isolates resistant to both INH and ethionamide were 19%[12].

In the present study 91% of M. tuberculosis isolates were resistant to clarithromycin. It is in agreement with other studies[13]. The role of clarithromycin in the treatment of M. tuberculosis is not very clear. When used alone it shows a high percentage of resistance but its use in combination needs to be investigated in view of the rising numbers of MDR tuberculosis. At present clarithromycin is used for M. avium complex (MAC). It has not been used for treatment of M. tuberculosis because of its high (MICs) in-vitro as tested by either conventional or Bactec methods. In most cases MIC was much higher than the concentrations achieved in serum[14]. In our study also higher MICs were observed with resistence ratio>8. Cavalieris et al has suggested synergistic activitivy of clarithromycin and first-line antituberculosis drugs against MDR-tuberculosis[15]. In their study addition of clarithromycin to INH, rifampicin and ethambutol resulted in 4 to 32 fold reductions of MICs of MDR strains. They concluded that the ability of clarithromycin/14 hydroxycla-rithromycin to enhance the activities of isoniazid, ethambutol, and rifampin in vitro suggests that this combination may be efficacious in the treatment of multidrug-resistant M. tuberculosis infections. However other investigators have not confirmed this synergistic activity. Clarithromycin has no antituberculosis activity in murine model of tuberculosis. Mor and Esfandiari have also demonstrated synerigistic activities of clarithromycin and pyrazinamide against M.

tuberculosis in human macrophages. MICs were four to eight fold lower for this combination than for either drug alone[16].

In our study we investigated clarithromycin alone. Clarithromycin,s MIC even against the sensitive control strain H37 Rv was high (MIC 16-32). This was in agreement with other studies. David et al have demonstrated synergistic activity of clarithromycin with cell wall inhibitors (bacitracin, vancomycin, and ethambutol). Clarithromycin,s resistance may be due to permeability barrier which can be weakened by use of cell wall inhibitors leading to enhanced activity of the drug. In such a combination this drug was even effective against MDR strains of M. tuberculosis[17]. This finding needs further research on use of clarithromycin as antituberculosis drug.

We found clarithromycin resistant in 91 % strains. However because of its synergistic effect it is recommended that it should be tested in combination with other antibiotics. Its role in future in the treatment of tuberculosis, specially the MDR strains cannot be ignored though it needs extensive research. Majority of M. tuberculosis strains were sensitive to the other drug in our study ethionamide. Therefore it may be used as an alternative drug when first line therapy fails. Sensitivity testing of MDR M. tuberculosis isolates against a broad range of antibiotics is recommended to design an appropriate treatment regimen.

REFERENCES

1. Brown P. A disease that is alive and kicking. World health 1993; 4: 4-5.
2. WHO, Tuberculosis Fact Sheet, March 2007
3. Karamat KA, Hayat S, Butt T, Abbasi S. Multidrug resistant tuberculosis. Pak Armd F Med J 2000; 50: 2: 114-6.
4. Kochi A. The global tuberculosis situtation and the newer control strategy of the world health organization. Tubercle 1991; 72:1-6. Reproduced, Bull World Health Organization 2001; 79: 1: 71-5.
5. Brodie D, Schulger NW. The diagnosis of tuberculosis. Clin Chest Med 2005; 26: 247-71.
6. Laidlaw M. Mycobacterium tubercle bacilli. In: Collee JG, Duguid JP, Frazer AG, Marmion BP eds. Mackie & Mc Cartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone. 1989: 399-424.
7. Clark B, Inderleid CB, Pfyffer GE. Susceptibility test methods: Mycobacteria. In: Maurray PR, Baron EJ, P faller MS, TenovrFC, Yolken RH, Eds. Mannual of Clinical Microbiology 8th Ed. Washington DC. ASM Press, 2003; 1149 – 77.
8. Pfyffer GE, BonatoDA, Ebrahimzadeh A, Gross W, Hotaling J, Kornblum J, et al. Multicentre laboratory validation of susceptibility testing of M. tuberculosis against classical second–line and newer antimicrobial drugs by using the radiometric Bactec 460 technique and the proportion method with solid media. J Clin Microbiol 1999;37:3179-86.
9. DeBarber AE, Mdluli K, Bosman M, BekkerLG, Barry CE. Ethionamide activation and sensitivity in multidrug-resistant Mycobacterium tuberculosis. Microbiology 2000; 97: 17: 9677-82
10. Heym B, Honore N, Truffot Pernot C, Banerjee A, Schurra C, Jacoles WR et al. Implications of multidrug resistance for the short course chemotherapy o tuberculosis: a molecular study. Lancet 1994; 344 (8919): 293–8.
11. Musser JM. Antimicrobial agents resistance in mycobacteria; Molecular genetic insights. Clin Microb Rev 1995; 496-514.
12. Fattorini L, Iona E, Ricci ML, Thoresen OF, Orru G, Oggioni MR et al. Activity of 16 antimicrobial agents against drug-resistant strains of Mycobacterium tuberculosis. Microb Drug Resist 1999; 5: 4: 265-70.
13. Pernot CT, Lounis, Grosset JH, Clarithromycin is inactive against Mycobacterium tuberculosis. Antimicrob agents chemother1995; 2827-8.s
14. Perri DG, Bonora S. Which agents should we use for the treatment of multidrug-resistant M. tuberculosis? J Antimicrob Chemother2004; 54; 593-602.
15. Cavalieri SJ, Biehle JR, Sander WE. Synergistic activities of clarithromycin and antituberculosis drugs against multidrug resistant M. tuberculosis. Antimicrob Agents Chemother 1995;1542-5.
16. Mor N, Esfandiari A. Synergistic activities of clarithromycin and pyrazinamide against M. tuberculosis in human macrophages. Antimicrob Agents Chemother 1997; 2035-6.
17. David SB, Barros V, Verde SC, Portugal C, David HL, Intrinsic resistance of M. tuberculosis to clarithromycin is effectively reversed by subinhibitory concentrations of cell wale inhibitors. J. Antimicrob chemother 2000; 46: 391-5.