In Vitro Activity of Linezolid Against Multidrug-Resistant Mycobacterium Tuberculosis Isolates By Bactec Mgit 960 Method

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ABSTRACT

• ***Objective:*** *The aim of our study was to determine susceptibility of linezolid to multi-resistant Mycobacterium tuberculosis isolates.*

• ***Material and Method:*** *Seventy two multi drug-resistant Mycobacterium tuberculosis isolates were studied under the activity of 1 µg/ml of linezolid by the BACTEC MGIT 960 susceptibility method.*

• ***Results:*** *Seventy two multi drug-resistant Mycobacterium tuberculosis species were determined to be susceptible to linezolid.*

• ***Conclusion:*** *Linezolid was found to be effective to multi-resistant Mycobacterium tuberculosis in vitro.*

• ***Key Words:*** *Multi drug-resistant Mycobacterium tuberculosis, linezolid, in vitro susceptibility* ***Nobel Med 2009; 5(1): 31-34***

ÖZET

BACTEC MGIT 960 YÖNTEMİYLE LİNEZOLİDİN ÇOKLU İLAÇ DİRENÇLİ MYCOBACTERIUM TUBERCULOSIS SUŞLARINA IN VITRO ETKİNLİĞİ

• **Amaç:** Bu çalışmanın amacı linezolidin çoklu dirençli *Mycobacterium tuberculosis* suşlarına olan duyarlılığını saptamaktır.

• **Materyal ve Metod:** Yetmiş iki çoğul ilaç-dirençli *Mycobacterium tuberculosis* suşunun linezolidin 1µg/ml oranındaki etkinliği BACTEC MGIT 960 duyarlılık yöntemiyle çalışıldı.

• **Bulgular:** Yetmiş iki çoklu dirençli *Mycobacterium tuberculosis* suşunun linezolide duyarlı olduğu saptandı.

• **Sonuç:** Linezolid in vitro olarak çoklu dirençli *Mycobacterium tuberculosis* suşlarına etkili olduğu sonucuna varıldı.

• **Anahtar Kelimeler:** Çoğul dirençli *Mycobacterium tuberculosis*, linezolid, in vitro duyarlılık. **Nobel Med 2009; 5(1): 31-34**

INTRODUCTION

The ongoing global burden of tuberculosis (TB) and the increasing problem of multidrug-resistant (MDR) TB (resistant to at least isoniazid and rifampin) have led to the increased use of second-line anti-TB drugs.[1] Treatment of patients infected with first-line-resistant organisms, including those resistant to rifampicin and isoniazid, constitutes a real clinical challenge. Moreover, the tuberculostatic agents against MDR *M. tuberculosis* isolates remains very limited.[2]

Oxazolidinones may have a place as anti-tuberculosis agents especially for the treatment of infections due to multi-drug resistant strains. Linezolid is a member of the oxazolidinone and its mechanism of action is inhibition of the protein synthesis.[3] The BACTEC MGIT 960 is a fully automated, high capacity, and non radiometric system, and it monitors 960 7 ml culture tubes.In order to monitor microbial growth, the BACTEC MGIT 960 uses the same oxygen-quenching fluorescent sensor technology and for the detection of *M. tuberculosis complex* is providing greater recovery.[4] The critical concentrations for linezolid in the MGIT 960 system is 1.0 µg/ml and the BACTEC MGIT 960 system is an accurate method for rapid testing of the susceptibility of *Mycobacterium tuberculosis* isolates to linezolid.[5]

The aims of the present study were to determine the in vitro activity of linezolid strains belonging to the MDR *M.tuberculosis complex* isolates by BACTEC MGIT 960 technique.

MATERIAL and METHOD

Bacterial Strains

The activity of linezolid was evaluated against 72 isolates of the MDR *M. tuberculosis*. Isolates were detected line probe assay (inno-lipa mycobacteria, innogenetics, Belgium) or automated culture methods (BACTEC MGIT 960, Becton dickinson, sparks,MD or BacT/Alert 3D, bioMèrieux) at the Tuberculosis units of the Ege University hospital and Izmir Atatürk Training and Research Hospital. *Mycobacterium tuberculosis* ATCC 27294 (H37Rv) isolate was used as controls.

Antimicrobial Agent

Linezolid was kindly provided by Pfizer Laboratories (Pharmacia, Istanbul, Turkey) in powder form with stated potency and was dissolved in deionized water. Linezolid solution was sterilized using a 0.22 µm polycarbonate fitler membrane, and the first 20% of the initial filtrate was discarded. Stock solution was stored at -70°C in small aliquots.

Culture System

BACTEC MGIT 960 drug susceptibility testing supplement 0.8ml (oleic acid-albumin-dextrose-catalase), 100 µl of the drug stock solution (1 µg/ml), and 0.5 ml of the suspension containing MDR *M. tuberculosis* were added to an MGIT tube. The growth

Table 1: Number of growth control reach to GU value of 400 and days.

Days	Number of growth control reach to GU value of 400 (%)
7	2 (2.8)
8	12 (16.7)
9	18 (25)
10	25 (34.7)
11	10 (13.9)
12	5 (6.9)

GU: Growth Unit

control tube did not contain linezolid. Growth control tubes were prepared for each isolate. Linezolid susceptibility testing sets were entered into the BACTEC MGIT 960 instrument and continuously monitored until a susceptible or resistant result was obtained.[6] For the drug susceptibility testing set (DST) containing "unknown drug" the instrument flagged the DST set "complete" when the growth control reached a growth unit (GU) value of 400. If the GU of the drug-containing tube was more than 100 when the GU of the growth control was 400, the results were defined as resistant. If the GU values of the drug-containing tubes were equal to or less than 100, the results were considered susceptible.[5]

Statistical Analysis

We evaluated the days of growth control reached a GU value of 400 using Pearson chi-square test. When p value was ⩽0.05, the results considered to be statistically significant. The analysis were performed with the SPSS 15.0 software (SPSS inc. Chicago, IL).

RESULTS

Mycobacterium tuberculosis ATCC 27294 (H37Rv) isolate and all the growth controls of the MDR *M. tuberculosis* isolates were recovered in the BACTEC MGIT 960 system. All the growth controls reached a GU value of 400 and all the MDR *M. tuberculosis* isolates were susceptible to 1µg/ml linezolid solutions. None of the 72 MDR M. tuberculosis isolates were resistant to linezolid. All the growth control reach to GU value of 400 between 7 and 12 days (a period of 4-12 days is recommended by the Becton Dickinson). Number of the growth control positive tubes on the 10th day was the highest. This was statistically significant ($p<0.05$). Number of growth control which reach to GU value of 400 and days are shown in Table 1.

DISCUSSION

TB remains a major public health problem worldwide. In recent years, the incidence of TB has been rising. There is also an emergence of MDR-TB which worsens the impact of this disease. The increasing prevalence of MDR-TB has greatly contributed to problems of TB such as the increasing rate of MDR-TB and the high rate of a co-infection with human immunodeficiency virus, The development of new potent anti-TB drugs without cross-resistance with known antimycobacterial agents is urgently needed.[7,8] MDR-TB is an increasing in many parts of the world and in Turkey. Recent studies have shown that MDR-TB rates were between 4.8%-8% in Turkey.[9-11] Oxazolidinones are a new class of totally syntetic antibacterial agents with wide spectrum of activity against a variety of clinically significant susceptible and resistant bacteria. This antibacterial has been shown to inhibit translation at the initation phase of protein synthesis and linezolid showed good activity against TB.[12] We used 1 µg/ml linezolid, Rüsch-Gerdes, et al. have determined that the critical concentrations for linezolid was 1 µg/ml.[5] We evaluated in vitro activity of linezolid against MDR-TB isolates by the BACTEC MGIT 960 method. Linezolid has shown good activity to all 72 isolates and all isolates were susceptible to it. Linezolid-resistant clinical TB strains seem to be rare. A previous study from Germany has declared a rate of 1.9% (4/210 isolates) resistance to linezolid. These four isolates have shown resistance to at least isoniazid, rifampin, streptomycin, and ethambutol and they were MDR-TB. No mutations have detected in potential target genes.[1] Another study found mutations in the 23SrRNA gene and G-to-T base pair exchange at different positions.[13] Until now, few studies have previously assayed the in vitro activity of linezolid against *M. tuberculosis* isolates by the BACTEC MGIT 960 or another methods. Tato, et al. studied 55 *M. tuberculosis* isolates including MDR-TB and *M. bovis* isolates and all of them were susceptible to linezolid.[2] Similary, Erturan, et al. have determined that linezolid was susceptible to all MDR-TB isolates.[14] In recent years significant increases in the MIC (90)s of linezolid in MDR-TB isolates were established; Richter, et al. have detected (first time) linezolid-resistant clinical isolates of TB for the first time and Huang, et al. have assinged that MIC (90)s of linezolid have changed from 0.5 µg/ml to 2 µg/ml during the period of 2001 to 2003 and three strains MIC were 4 µg/ml.[1, 15] Linezolid also has good in vivo activity against TB, daily-half doses of linezolid were effective in patients with MDR-TB but side effects can be seen such as peripheral and optic neuropathy,and bone marrow depression.[16, 17]

BACTEC MGIT 960 system is a useable method for sensitivity testing to TB isolates and it has been shown to work well.[4-6] We determined a growth control which reached to GU value of 400 between 7 and 12 days. A period of seven days is a good time for the recovery of TB isolates, and we can determine the results of susceptibility in maximum two weeks.

CONCLUSION

In conclusion, our study demonstrates that linezolid has a good in vitro activity against MDR-TB isolates and BACTEC MGIT 960 system is a reliable method for testing the susceptibility of TB.

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