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THE EFFECT OF FLUOROQUINOLONES, METRONIDAZOLE AND SOME ANTIFUNGALS ON *MYCOBACTERIUM TUBERCULOSIS* (H37Rv)

MYCOBACTERIUM TUBERCULOSIS (H37Rv)'E FLROKİNOLONLAR, METRONİDAZOL VE BAZI ANTİFUNGALLERİN ETKİSİ

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Anahtar Sözcükler: *Mycobacterium tuberculosis, in vitro* antimikrobiyal duyarlılığı, florokinolonlar, metronidazol, antifungaller, mikrodilüsyon yöntemi

SUMMARY

The purpose of this study was to assess the antituberculosis potential of some drugs including metronidazole, fluconazole, econazole, miconazole, clotrimazole, ketoconazole, amphotericin B, 5-fluorocytosine in comparison to well-known primary and secondary (flouroquinolones) antituberculosis agents. The MIC values were determined with broth microdilution method. The MIC values for Mycobacterium tuberculosis H37Rv of isoniazid, rifampicin, streptomycin and ethambutol (primary antituberculosis agents) were 0.125 μg/ml, <0.06 μg/ml, 2 μg/ml and 4 μg/ml, respectively. Ofloxacin, ciprofloxacin, pefloxacin, metronidazole, clotrimazole, and ketoconazole of MIC values were 0.5 μg/ml, 1 μg/ml, 4 μg/ml, >256 μg/ml, 16 μg/ml and 16 μg/ml, respectively. The results of the study showed that some antimicrobial agents particularly clotrimazole and ketoconazole have antituberculosis activity and they should be studied further to determine their in vivo effect in tuberculosis.

ÖZET

Bu çalışmanın amacı; metronidazol, flukonazol, ekokonazol, mikonazol, klotrimazol, ketokonazol, amfotersin B, 5-florositozin gibi bazı ilaçların antitüberküloz etkilerini incelemek ve bu etkileri birincil antitüberkülozlar ve ikincil antitüberkülozların etkileri ile karşılaştırmak idi. Araştırmada, Mycobacterium tuberculosis (H37Rv) suşu ve buyon mikrodilüsyon yöntemi kullanıldı. Birincil antitüberküloz ilaçların (izoniazit, rifampisin, streptomisin ve etambutol) MIC değerleri, sırasıyla, 0.125 μg/ml, <0.06 μg/ml, 2 μg/ml ve 4 μg/ml olarak bulundu. Ofloksasin, siprofloksasin, pefloksasin, metronidazol, klotrimazol ve ketokonazole ilişkin MIC değerleri ise, sırasıyla, 0.5 μg/ml, 1 μg/ml, 4 μg/ml, >256 μg/ml, 16 μg/ml ve 16 μg/ml olarak saptandı. Sonuç olarak, özellikle klotrimazol ve ketokonazol olmak üzere, bazı ilaçlar in vitro antitüberküloz etki göstermektedirler. Bu ilaçların in vivo antitüberküloz etkileri başka çalışmalarla incelenmelidir.

INTRODUCTION

Mycobacterium tuberculosis, which causes tuberculosis, is the greatest single infectious cause of mortality worldwide, killing roughly two million people annually. Estimates indicate that one-third of the world population is infected with latent *M. tuberculosis*. The synergy between tuberculosis and the AIDS epidemic, and the

surge of multidrug-resistant clinical isolates of *M. tuberculosis* have reaffirmed tuberculosis as a primary public health threat (1).

The introduction of specific chemotherapy for tuberculosis approximately 50 years ago accelerated the decline in the incidence of the disease in industrialized countries. In the past few years that decline has been arrested, and in some populations there has been an alarming increase in tuberculosis (2). The recent emergence of outbreaks of multidrug-resistant tuberculosis (MDR-TB) outbreaks pose serious threat to the treatment of the disease. Thus, there is an urgent need to develop new tuberculosis drugs that are active against MDR-TB strains (3).

A principle target of flouroquinolones is thought to be DNA gyrase, the enzyme responsible for controlling negative supercoiling in bacterial DNA. During the supercoiling reaction, gyrase transiently breaks duplex DNA, and the flouroquinolone traps a reaction intermediate containing broken DNA. That process leads to cell death when the DNA breaks are released from gyrase mediated constraint. Two fluoroquinolones, ciprofloxacin and ofloxacin, are currently used as antituberculosis agent (4).

Antifungals are drugs that increase membrane permeability and inhibit synthesis of sterols (ergosterol) in fungal cell membranes among other actions (5).

Metronidazole is an antiprotozoal drug used in treating *Trichomanas, Giardia,* and amebic infections. It also has striking effect on anaerobic bacterial infections, eg, those due to *Bacterioides* spp., and bacterial vaginosis. It appears to be effective for the preoperative preparation of the colon and in antibiotic-associated diarrhea caused by toxigenic Clostridium difficile (5). In addition, metronidazole was found to be able to kill dormant cells of *M. tuberculosis* (6).

Antituberculosis activity was determined by broth microdilution method using 96 well-microplates, because Yamane et al. (7, 8) and Leite et al. (9) reported that the newly developed microdilution test method for *M. tuberculosis* is a practical, rapid, quantitative, nonradiometric alternative for the determination of MICs in clinical mycobacteriology laboratories. Yamane (10) also explained that their procedure (broth microdilution method) is very close to the guidelines of Centers for Disease Control and Prevention (CDC), Atlanta, USA (1994), except for isolation and identification of *M. tuberculosis*.

The purpose of this study was to assess the antituberculosis potential of some drugs including metronidazole, fluconazole, econazole, miconazole, clotrimazole ketoconazole, amphotericin B, 5-fluorocytosine in comparison to well-known primary and secondary antituberculosis agents.

MATERIAL AND METHOD

Strains

Antituberculosis activity against *M. tuberculosis* (H37Rv) was tested with broth microdilution method using 96 well-microplates (9, 11).

Antimicrobial agents

Isoniazid, rifampicin, streptomycin, ethambutol, and antifungals were purchased from Sigma. Metronidazole was provided by Eczacıbaşı (Istanbul). A stock solution for each compound was prepared according to CDC (1985) recommendation and used at 0.06 to 256 µg/ml concentrations (12).

Inoculum preparation

Colonies were scraped from a freshly growing (3 to 4 weeks) Loewenstein-Jensen medium slant into 3 ml of Middlebrook 7H9 broth containing four to five 3-mm-diameter glass beads in a conical tube. The tubes were vortexed vigorously for 3 to 5 min to homogenize the suspension. The large particles were allowed to settle, and supernatant was adjusted to turbidity equivalant to a 0.5 Mc Farland (13-16).

Preparation of Minimum Inhibitory Concentration (MIC) plates

96-well microtitre plates with U-shapped wells were used. The plates were arranged to give 12 rows by eight lanes and these were then filled with 0.1 ml amounts of Middlebrook 7H9 medium, supplemented with OADC enrichment. The stock suspensions of drugs were diluted in Middlebrook 7H9 medium and serial dilution for each drug were prepared and 0.1 ml volumes were dispensed into plates (9, 11).

Inoculation

Each well was inoculated with 0.01 ml of bacterial suspension (0.5 Mc Farland standard). Medium without antimicrobial agents was inoculated with the same suspension and with a 100 fold diluted suspension, as a growing control. The plates were sealed, put in plastic bags and incubated 37°C for 28 days in a moisturized incubator. The MIC values were recorded on days 7, 14 and 21. (9, 11). Slides were prepared from each well for acid-fast staining. No organisms other than acid-fast bacilli were observed.

RESULTS

The results are summarized in Table 1. MIC values of ofloxacin, ciprofloxacin, pefloxacin, clotrimazole and ketoconazole were 0.5 µg/ml, 1 µg/ml, 4 µ/ml, 16 µg/ml, 16 mg/ml, respectively.

The results of the study showed that among some antimicrobial agents particularly clotrimazole and ketoconazole have antituberculosis activity.

Tablo 1. MIC values of some antimicrobials against *M. tuberculosis* (H37Rv)

Antimicrobials	MIC (µg/ml)	Antimicrobials	MIC (µg/ml)
Isoniazid	0.125	Fluconazole	>256
Rifampicin	<0.06	Econazole	>256
Streptomycin	2	Miconazole	>256
Ethambutol	4	Clotrimazole	16
Ofloxacin	0.5	Ketoconazole	16
Ciprofloxacin	1	Amphotericin B	>256
Pefloxacin	4	5-fluorocytosine	>256
Metronidazole	>256		

DISCUSSION

The best tuberculosis therapy is with isoniazid, rifampicin, pyrazinamide and ethambutol in the form of DOTS (Directly Observed Treatment, short-course) recommended by the World Health Organization for treating every tuberculosis patient and takes a lengthy period of six months (3). In the mean time, new drugs are sought for the control of tuberculosis because resistance to classical antituberculosis drugs has dramatically increased (17).

Fluoroquinolone drugs, such as ciprofloxacin, ofloxacin, and sparfloxacin, are active *in vitro* against *M. tuberculosis* isolates and have been demonstrated to be effective in the treatment of tuberculosis (17-21). In this study, the MIC values for ciprofloxacin, ofloxacin and pefloxacin against *M. tuberculosis* (H37Rv) were found to be 1 µg/ml, 0.5 µg/ml, 4 µg/ml, respectively.

Metronidazole was chosen for the present study as it is known to be selectively active against anaerobes, and the shift-down to dormancy of *M. tuberculosis* represents a metabolic adaptation to survival under condition of severe O2 depletion, presumably with a low order of anaerobic maintenance metabolism (2, 3, 6, 22). Although metronidazole has been reported to be able to kill dormant cells of *M. tuberculosis*, no effect (MIC value was >256 µg/ml for metronidazole) was observed against the active form of *M. tuberculosis* (H37Rv) strain.

Sun and Zhang (3) reported that MIC values of miconazole, 2-nitroimidazole, 4-nitroimidazole, clotrimazole and thiabendazole for *M. tuberculosis* (H37Ra) strain were 2 µg/ml, 5-10 µg/ml, 20 µg/ml, 2-5 mg/ml, 20 µg/ml, respectively. In the present study, the MIC values of clotrimazole and ketoconazole for *M. tuberculosis* (H37Rv) strain were 16 mg/ml. Antifungal imidazoles increase membrane permeability and inhibit synthesis of sterols (ergesterol) in fungal cell membranes (5). *Mycobacterium tuberculosis* cell wall is rich in lipid molecules (16). Therefore, clotrimazole and ketoconazole might effect lipid synthesis of the *M. tuberculosis* cell wall.

The results of the present *in vitro* study clearly showed that among some antimicrobial agents particularly clotrimazole and ketoconazole exert antituberculosis activity and that they should be studied further for their effectiveness against tuberculosis *in vivo*.

REFERENCES

1. **Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, et al.** A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* **2000;** 405: 962-6.
2. **Wayne LG, Sramek HA.** Metronidazole is bactericidal to dormant cells of *Mycobacterium tuberculosis. Antimicrob Agents Chemother* **1994;** 38: 2054-8.
3. **Sun Z, Zhang Y.** Antituberculosis activity of certain antifungal and antihelmintic drugs. *Tuberc Lung Dis* **1999;** 79: 319-20.
4. **Xu C, Kreiswirth BN, Sreevatsan S, Musser JM, Drlica K.** Fluoroquinolone resistance associated with specific gyrase mutations in clinical isolates of multidrug-resistant *Mycobacterium tuberculosis. J Infect Dis* **1996;** 174: 1127-30.
5. **Brooks GF, Butel JS, Ornston LN.** *Medical Microbiology.* 19th ed. California: Prentice-Hall International, Inc; **1991:** 150, 178.
6. **Deidda D, Lampis G, Fioravanti R, Biava M, Porretta GC, Zanetti S, Pompei R.** Bactericidal activities of the pyrrole derivate BM212 against multidrug-resistant and intramacrophagic *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* **1998;** 42: 3035-7.
7. **Yamane N, Ichiyama S, Kawahara S, Iinuma Y, Saitoh H, Shimojima M, et al.** Multicenter evaluation of broth microdilution test, BrothMIC MTB, to determine minimum inhibitory concentrations (MICs) of antimicrobial agents for *Mycobacterium tuberculosis*-evaluation of interlaboratory precision and interpretive compatibility with agar proportion method. *Rinsho Byori* **1999;** 47: 754-66.
8. **Yamane N, Nakasone I, Saitoh H, Kaneda M, Shimojima M, Yamashita K, et al.** Evaluation of a newly developed broth microdilution test method to determine minimum inhibitory concentrations (MICs) of antimicrobial agents for mycobacteria. *Rinsho Byori* **1998;** 46: 719-27.
9. **Leite CQ, Beretta AL, Anno IS, Telles MA.** Standardization of broth microdilution method for *Mycobacterium tuberculosis. Mem Inst Oswaldo Cruz* **2000;** 95: 127-9.

10. **Yamane N.** Mycobacteria-methods to meet the CDC guidelines. *Rinsho Byori* **1999;** 47: 539-46.

11. **Wallace RJ, Nash DR, Steele LC, Steingrube V.** Susceptibility testing of slowly growing mycobacteria by a microdilution MIC method with 7H9 broth. *J Clin Microbiol* **1986;** 24: 976-981.

12. **Centers for Disease Control (CDC).** *Public Health Mycobacteriology. A Guide for the Level III Laboratory*. Atlanta, Georgia: US Department of Health and Human Service, **1985:** 159-84.

13. **National Committee for Clinical Laboratory Standards.** *Antimycobacterial Susceptibility Testing for Mycobacterium tuberculosis*. Tentative Standard M24-T. Villanova, PA: National Committee for Clinical Laboratory Standards, **1994.**

14. **Kent PT, Kubica GP.** *Public Health Mycobacteriology: A Guide for the Level III Laboratory*. Atlanta, Georgia: Public Health Service Centers for Disease Control, **1985.**

15. **Inderlied CB, Salfinger M.** Antimicrobial agents and susceptibility tests: Mycobacteria. *In:* Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of Clinical Microbiology*. 6th ed. Washington, DC: ASM Press, **1995:** 1385-404.

16. **Saniç A, Çoban AY.** *Mikobakteriler ve Laboratuvar Tanı*. Samsun: Otak AŞ Basımevi, **1999.**

17. **Kocagoz T, Hackbarth CJ, Unsal I, Rosenberg EY, Nikaido H, Chambers HF.** Gyrase mutations in laboratory-selected fluoroquinolone-resistant mutants of *Mycobacterium tuberculosis* H37Ra. *Antimicrob Agents Chemother* **1996;** 40: 1768-74.

18. **Chen CH, Shih JF, Lindholm-Levy APJ, Heifets LB.** Minimal inhibitory concentrations of rifabutin, ciprofloxacin, and ofloxacin against *Mycobacterium tuberculosis* in patients in Taiwan. *Am Rev Respir Dis* **1989;** 140: 987-9.

19. **Lalande V, Truffot-Pernot A, Paccaly-Moulin A, Grosset J.** Powerful activity of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in mice. *Antimicrob Agents Chemother* **1993;** 37: 407-13.

20. **Tomioka H, Sato K, Kajitani H, Akaki T, Shishido S.** Comparative antimicrobial activities of the newly synthesized quinolone WQ-3034 levofloxacin, sparfloxacin, and ciprofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. *Antimicrob Agents Chemother* **2000;** 44: 283-6.

21. **Ruiz-Serrano MJ, Alcala L, Martinez L, Diaz M, Marin M, Gonzalez-Abad MJ, Bouza E.** *In vitro* activities of six fluoroquinolones against 250 clinical isolates of *Mycobacterium tuberculosis* susceptible or resistant to first-line antituberculosis drugs. *Antimicrob Agents Chemother* **2000;** 44: 2567-8.

22. **Brooks JV, Furney SK, Orme IM.** Metronidazole therapy in mice infected with tuberculosis. *Antimicrob Agents Chemother* **1999;** 43: 1285-8.