In Vitro Efficacy of Fusidic Acid on Drug Resistant Strains of Mycobacterium Tuberculosis

Dirençli Tüberküloz Suşlarında Fusidik Asidin İn Vitro Etkinliği

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ABSTRACT

Introduction: We aimed to investigate the in vitro efficacy of fusidic acid (FA) on tuberculosis strains resistant to antituberculous drugs .

Material and Method: In our single center, prospective, randomized controlled study, we researched the efficacy of sodium fucidate on tuberculosis bacilli at dosages of 128-64-32-16 mg/l, carried out on Lowenstein Jensen solid media using absolute concentration method. Between March 2000 and February 2001, sputum cultures of 728 tuberculosis patients were tested for drug susceptibility against 4 major antituberculous drugs [isoniazid (H), rifampin (R), ethambutol (E), streptomycin (S)] including fusidic acid (FA). Firstly, the study was designed to determine drug susceptibility at 32-64-128 mg/l for fusidic acid. Out of 728 cultures 488 cultures were tested for these concentrations for fusidic acid and to 4 major drugs. Following this, additional 240 cultures were tested for 16mg/l concentration of fusidic acid and to major drugs.

Results: Drug susceptibility testing showed no resistance to the sodium fucidate at the dosages of 128-64-32 mg/l on 488 cultures. Resistance to R,H,S,and E were 10.4%, 8.19%, 5.32%, and 0.6% respectively. At 16 mg/l dosage the resistance to sodium fucidate was determined on 10 cultures (4.16%). Multi drug resistance rate was 7.5% among 728 cultures. There was sodium fucidate resistance in only one of the 55 multi drug resistant tuberculosis (MDR-TB) strains.

Conclusion: We determined that sodium fucidate has a powerful in vitro efficacy at dosages of 32 mg/l and over. It was also effective at dosage of 16 mg/l, but resistance was observed at this concentration. Fusidic acid could be an alternative drug for multidrug or extensively drug resistant cases.

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Key words: Fusidic acid, resistant strains, Lowenstein Jensen medium, drug sensitivity test

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ÖZET

Giriş: Sodyum fusidatın antitüberküloz ilaçlara dirençli tüberküloz suşlarında invitro etkinliğini araştırmayı amaçladık.

Gereç ve Yöntem: Tek merkezli, prospektif, randomize kontrollü bu çalışmada Mart 2000 ve Şubat 2001 tarihleri arasında hastanemizde Löwenstein Jensen katı besiyerinde kültür pozitifliği saptanan 728 hastada diğer antitüberküloz ilaçlarla birlikte fusidik asitin sodyum tuzu olan, sodyum fusidatın absolü konsantrasyon yöntemiyle invitro etkinliğini araştırdık. İlk etapta 728 kültürün 488' inde yapılan ilaç direnç çalışmasında 4 majör antitüberküloz ilaçla (izoniazid (H), rifampisin (R), etambutol (E), streptomisin (S)) birlikte, sodyum fusidatın 128-64-32mg/l dozlarında etkinliğini araştırdık. Daha düşük dozdaki etkinliğini görmek açısından 240 kültür pozitif suşta 16mg/l sodyum fusidatla aynı çalışmayı yaptık.

Bulgular: 128-64-32mg/l dozlarda sodyum fusidata hiç bir suşta direnç saptanmadı. Diğer ilaç dirençleriyse; H %8.19, R %10.4, S %5.32, E %0.6 olarak saptandı. 16 mg/ml dozda ise 10 suşta (%4.16) sodyum fusidat direnci saptandı. 728 kültürde saptanan çok ilaca direnç oranı %7.5 idi ve 55 çok ilaca dirençli tüberküloz (MDR-TB) suşundan sadece birinde sodyum fusidat direnci vardı.

Sonuç: Fusidik asitin 32mg/l ve üstündeki dozlarda MDR-TB suşları da dahil olmak üzere güçlü bir invitro etkinliğinin olduğunu, 16mg/l dozda da etkili olduğunu ancak direnç probleminin doğabileceğini saptadık. Fusidik asit çok ilaca dirençli vakalarda alternatif bir ilaç olabilir.

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Anahtar sözcükler: Fusidik asit, direçli suşlar, Löwenstein Jensen besiyeri, ilaç direnç testi

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INTRODUCTION

Tuberculosis (TB) is one of the world's leading causes of death. Although TB incidence tended to decrease in high income countries after implementing anti TB chemotherapy in the 1950s, it is still a serious health problem in developing nations [1,2].

About one third of the world's population is infected by M. tuberculosis. In our country the TB incidence rate

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is about 30 in one hundred thousand population and nearly 20-30 thousand new cases develop active disease each year [3].

Multi drug resistant tuberculosis (MDR-TB) was defined in the early 1990s. Strains of M. tuberculosis that are resistant to both H and R with or without resistance to other drugs have been defined as multidrug resistant strains. H and R are the most essential drugs in the management of TB. Resistance to both drugs in the more severe form of TB, developing due to the misuse or interruption of TB treatment, is usually no longer treatable with first line drugs. Second line drugs are used for the treatment of MDR-TB. In many studies flouroquinolones proved to be effective against MDR bacilli, but there is still a need for new drugs in the event of resistance to quinolones. For this purpose, the efficacy of the drug groups to drug resistant TB bacilli such as flouroquinolones, beta-lactamase resistant antibiotics, and aminoglycosides, has been assessed in many studies.

In this study we aimed to investigate the efficacy of a new drug not presently used in TB treatment. This drug is fusidic acid, is a member of the fucidan class obtained from Fusidium coccineum fungus. Although it has a steroid-like structure, it does not have steroidal activity. It has a Cephalosporin P-like structure. The sodium salt of fusidic acid has been developed in the Leo laboratory in Denmark in 1962 and since then it has been used clinically to treat staphylococcal infections of skin, soft tissue, eyes, bones and the lower respiratory tract [4,5].

MATERIAL AND METHOD

A total of 728 patients with culture positive tuberculosis who had been referred to our hospital, which is a reference and education center for lung diseases and tuberculosis, were included in our study. Sputum samples were homogenized for bacteriological examination. Smear positive samples were cultured on Lowenstein- Jensen media and tested for drug sensitivity with absolute concentration technique simultaneously. Smear negative samples tested for drug resistance after growth were determined on cultures. The concentrations for the drugs used in the culture medium were as follows: H (1 mg/l), S (10 mg/l), R (40 mg/l), E (2 mg/l), Sodium fucidate (16-32-64 and 128 mg/l). The evaluation was performed after keeping in an incubator at 37 degree for approximately five weeks.

Between March 2000 and November 2000, the samples of 488 tuberculosis cases were tested for drug sensitivity to H, R, E, S and fusidic acid. The concentrations for fusidic acid used in the cultures were 32, 64 and 128 mg/l. Between November 2000 and February 2001, an additional 240 cultures were tested for 16mg/l concentration of FA. Resistance to any drugs during drug susceptibility tests was accepted as positive when the number of colonies growing on the medium was >10.

RESULTS

Drug susceptibility tests showed no resistance to the sodium fucidate at the dosages of 128-64-32 mg/l on 488 cultures. The resistance to sodium fucidate was determined on 10 (4.16%) out of 240 cultures at 16 mg/l dosage (Table 1).

Overall resistance rates to R, H, S and E were 10.4%, 8.19%, 5.32%, and 0.6% respectively. The multi drug resistance rate was 7.5% among 728 cultures. There was sodium fucidate resistance in only one of the 55 MDR-TB strains (Table 2).

Also in one strain there was resistance to rifampicin together with the resistance to fusidic acid. The remaining 8 resistances to fusidic acid were resistant to only one drug.

DISCUSSION

Na-fucidate, the sodium salt of fusidic acid, is an important agent used clinically against staphylococcus and other Gr (+) bacteria since 1962. It is accepted as it has a narrow spectrum. To date, the staphylococcal efficacy of this drug is much better known than its antituberculosis activity because tuberculosis has been treated with the major tuberculosis drugs [6].

The confusing subject in these studies was the different concentrations of fusidic acid and declared different minimum inhibitory concentration (MIC) values and efficacies.

Fuursted et al. investigated the activity of fusidic acid; on 40 materials with M. tuberculosis (20 of them are resistant to one or more than one drugs) and 10 materials with M. bovis in BACTEC. Both the minimal inhibitor and the minimal bactericidal concentrations were determined (Table 3). Minimal bactericidal concentration was defined as the concentration that killed more than 99% of the bacteria [7].

Fuursted et al. in the same study; used 32 mg/l fusidic acid combined with; E (7.5 mg/l), H(1 mg/l), R(2 mg/l), and S (6 mg/l) consecutively. They declared that there was no synergism or antagonism when fuscidic acid was combined with standard antituberculosis drugs against

Table 1. Rates of fusidic acid resistant cultures

	Fusidic acid dosages (mg/ml) 32-64-128 mg/ml (first group)	Fusidic acid dosages (mg/ml) 16 mg/ml (second group)
Number of cultures	488	240
Fusidic acid resistant cultures number (%)	0 (0%)	10 (4.16%)

Table 2. Rates of fusidic acid resistant MDR cultures

Fusidic acid dosages	Number of multi drug resistant cultures	Rates of fusidic acid resistance Number (%)
32-64-128 mg/ml	34	0 (0)
16 mg/ml	21	1 (4.76)

drug resistant strains. However, in another study by Hoffner et al., a synergy between fusidic acid and e thambutol was found and there was no difference between fusidic acid dosages of 125 mg/l and 500 mg/l. Therefore we did not use any dosage over 128 mg/l in our study.

Hoffner et al., have tested in vitro efficacy of fusidic acid at the dosages of 32-64 mg/l on the 30 M. tuberculosis cases [8]. This study has become much more important because 11 of 30 cases were resistant to drugs and 6 of them had HIV infection. The minimal inhibitory concentrations in 30 cases were shown in Table 4.

The concentrations of 34-64 mg/l used in our study are compatible with this study and appears to support our results. In vitro activity of fusidic acid on sputum cultures of TB patients with HIV infection has been investigated by Hoffner et al. [8]. No significant difference has been determined between the efficacy of fusidic acid and required MIC concentration among the groups of TB patients with or without HIV infection. There was no HIV positive patient in our study group.

Regarding the studies mentioned above; we concluded that the dosages of 32-64-128 mg/l that we chose were not very high. In fact there are written sources notifying that by taking the normal dosage of 3 x 500 mg of fusidic acid orally, the serum concentration could reach 80-100 mg/l. Although the serum concentrations taken under medical treatment have been defined different in various publications [5,9-11] serum values were also related to accumulating concentrations of the drug in the body during the treatment period.

In the publication of Van Caekenberghe, the MIC 50% value of fusidic acid for M. tuberculosis has been determined as 16 mg/l. But the MIC value of fusidic acid on atypical mycobacteria strains ranged between 32-128 mg/l dosages [12].

Table 3. The in vitro activity of fusidic acid on 50 mycobacteria (7)

	Number	MIC90(range) (mg/l)	MBC90(range) (mg/l)	Range
M. tuberculosis Resistance (-)	20	16 (8-32)	250 (32-500)	32-500
M. tuberculosis Resistance (+)	20	16 (16-32)	250 (64-500)	64-500
M. Bovis	10	32 (16-32)	500 (125-500)	125 500

Abbrevations
MIC : minimum inhibitory concentration
MBC : minimum bactericidal concentration

Table 4. Inhibitory concentrations of fusidic acid dosages (10)

	Number of patients	Fusidic acid dosages (mg/l) 16 mg/l	32 mg/l	64 mg/l
M. tuberculosis sensitive to drugs	19	-	17	2
M. tuberculosis resistant to drug	11	1	9	1

In the study conducted by Kurt Fuursted et al. MIC of 32 mg/l was effective in M. tuberculosis. The dosages of 8-16 mg/l were adequate in the publication of Van Caekenberghe. Obtaining low resistance results at the dosages of 8-16 mg/l, which was chosen in other studies, could be an explanation for low resistance development in the dosages that we chose [13]. The reason why we studied with these higher dosages;was the notifying of growth at levels over the 64 mg/l in some publications, as well as the study conducted by Fabry et al.

The study of Fabry W. et al. is compatible with dosage selection in our study. Fabry used fusidic acid at the dosage of 8-32-64-128 mg/l on Lowenstein- Jensen culture medium and proportion dilution method. He used the E-test method for the same dosages and compared the two methods. Finally, he determined that the dosage of 64 mg/l, which was used in either dilution test or E-test; caused inhibition on 16 of 20 cultures, the remaining 4 cases were inhibited at the dosages of more than 64 mg/l, less than 128 mg/l. the inhibition under the 32 mg/l was established in 10 cultures [14].

The main reason for choosing the higher dosages of FA in our study is the fact that the serum concentration of 100 mg/l of FA could be achieved when fusidic acid is given in normal oral dosages (3 x 500 mg).

Resistance developed between 0-2% against drugs used for staphylococcus infections in long-term treatment [15]. It was a result of single-step mutation and its frequency is nearly one in a hundred thousand. Kurt Fuursted has determined the resistance as $1,7 \times 10^{-8}$ in his study. In fact, however, as fusidic acid will be used with at least four drugs in tuberculosis, the risk of resistance development is low [7,16,17].

The drug resistance rates to first line drugs (8.9% for H, 10.45% for R, 5.32% for S, 0.6% for E) were similar to the drug resistance rates of other studies in our country. Only the E resistance rates in our laboratory were lower than the other studies.

Tahaoglu et al. found the primary and secondary drug resistance rates in 1992 as being: for H 5.1% and 30%, for R 10.8% and 36.2%, for S 20.6% and 31.9%, and for E 4.2% and 11,2% respectively [18,19].

Our hospital is a reference center for TB and MDR-TB patients. The resistance rates determined in our laboratory do not reflect the rates of the population. as the most severe cases are examined in our hospital. In conclusion, fusidic acid has a significant antituberculous effect at the concentration of 32 mg/l and over as a second line drug in the treatment of MDR-TB. At the concentration of 16 mg/l, resistance may develop and the drug would be ineffective for the treatment.

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