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Original Article

EFFECTS OF ANTITUBERCULOUS DRUGS AND THEIR COMBINATIONS ON HUMAN POLYMORPHONUCLEAR LEUKOCYTE FUNCTIONS IN VITRO

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Abstract

Today new antibuberculous drugs and new combination regiments have been devevoped against the tuberculous resistance. However, the effects of these drugs on the immune system of the host has not been known yet. The aim of this study was to investigate the in vitro effects of primary antituberculous drugs [isoniazid (INH, 5µg/ml), rifampicine (RIF, 7µg/ml), pyrazinamide (PZA, 40µg/ml), ethambutol (EMB, 7µg/ml), streptomycine (S, 25µg/ml)], secondary antituberculous drugs [amikacin (A, 24µg/ml), ofloxacin (OFLX, 2,9µg/ml), cyclocerine (CYC, 10µg/ml), para-aminosalicilic acid (PAS, 90µg/ml), prothionamide (PTH, 1,6µg/ml), levofloxacin (LVFX, 2,8µg/ml)] and their combinations at therapeutic concentrations on polymorphonuclear leukocyte (PMN) functions (phagocytic and intracellular killing activity) of 15 healthy young volunteers, whose average age was 25.

PMNs were isolated by ficoll-hypaque gradient centrifugation method from venous blood with EDTA (0.1g/ml). Phagocytosis and intracellular killing activity were assayed by modifying Alexander's method.

A and PAS, which are secondary drugs that significantly increased the phagocytic activity, OFLX significantly increased the intracellular killing activity when compared with the control(drug-free) ($p<0.05$). The other primary and secondary drugs and their combinations did not significantly affect the phagocytic and intracellular killing activity when compared with the control(drug-free) ($p>0.05$).

As a conclusion, the use of antituberculous drugs and their combinations whose possitive effects are known not only on the microorganism but also on the immune system maybe useful in the treatment of patients with tuberculosis by showing stimulatory effect on PMN functions .

Key words: *Polymorphonuclear leukocyte, antituberculous drugs, phagocytosis, intracellular killing activity*

Antitüberküloz İlaç Ve Kombinasyonlarının İnsan Polimorf Nüveli Lökosit Fonksiyonları Üzerine İn Vitro Etkisi

Günümüzde tuberküloz direncine karşı yeni antitüberküloz ilaçlar ve yeni kombinasyonları geliştirilmektedir. Ancak bu ilaçların konağın immün sistemi üzerine olan etkileri henüz bilinmemektedir.

Çalışmamızda primer antitüberküloz ilaçlar [izoniazid (İNH, 5µg/ml), rifampisin (RiF, 7µg/ml), pirazinamid (PZA, 40µg/ml), etambutol (EMB, 7µg/ml), streptomicine (S, 25µg/ml)], sekonder antituberkulous ilaçların [amikasin (A, 24µg/ml), ofloksasin (OFLX, 2,9µg/ml), sikloserin (CYC, 10µg/ml), para-aminosalisilik asid (PAS, 90µg/ml), protionamid (PTH, 1,6µg/ml), levofloxacin (LVFX, 2,8µg/ml)] ve klinik kombimasyonlarının terapötik kombinasyonlarda, polimorf nüveli lökosit (PNL) fonksiyonları (fagositik ve hücre içi öldürme aktivitesi) üzerine etkisi yaş ortalaması 25 olan 15 sağlıklı gençte in vitro araştırılması amaçlanmıştır. PNL'ler EDTA'lı venöz kandan Ficoll-Gradient yöntemi ile ayrılmıştır. Fagositoz ve hücre içi öldürme aktivitesi tayininde Alexander ve arkadaşlarının yöntemi modifiye edilerek kullanılmıştır.

Sekonder ilaçlardan A ve PAS kontrole göre fagositik aktiviteyi anlamlı artırırken OFLX hücre içi öldürme aktivitesini anlamlı artırmıştır ($p<0.05$). Diğer primer ve sekonder antituberküloz ilaçlar fagositik ve hücre içi öldürme aktivitesini anlamlı olarak değiştirmemiştir ($p>0.05$).

Sonuç olarak, etkene olduğu kadar immün sistem üzerine de olumlu etkileri bilinen antituberküloz ilaç ve kombinasyonlarının kullanılması PNL fonsiyonları üzerinde stimülatör etki göstererek tüberkülozlu hastaların tedavisine katkıda bulunabilir.

Anahtar Kelimeler: *Polimorfonüklear lökosit, antitüberküloz ilaçlar, fagositoz, intraselüler öldürücü aktivite*

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INTRODUCTION

Neutrophils and macrophages are usually the most common cells of the immun system to encounter the invaders such as bacteria and fungi. The cells can easily have the circulation and attack the intruder at any place any time. The neutrophil responce to infection in vivo is iniated by adherence of neutrophils to vascular endothelial cells and progresses to the directed migration of neutrophils in to the extravascular tissue space (1,2).

Mycobacterial pathogens present healthy treatment worldwide. Mycobacterium tuberculosis alone accounts for 8-10 million new cases of pulmonary tuberculosis (TB) and approximately 3 million deaths each year (WHO, 1996) . Available statistics indicate that there is a close relationship between acquired immunodeficiency syndrome (AIDS) and tuberculosis (3,4).

Primary drugs chemotherapy against tuberculosis began in the late 1940's with streptomycine (S) closely followed by p-aminosalicilic acid (PAS) which is rarely used today and the key drug isoniazid (INH) while rifampicin (RIF), the other key drug was introduced in the late 1960's pyrazinamide (PZA) which was known already in the 1950's but caused side effects in the dosages when introduced as short course chemotherapy and given in lower doses. In the 1970's ethambutol (EMB) was mainly an adjunct drug preventing development of resistance (5).

Recently, secondary antituberculous drugs were introduced including quinolones such as ciprofloxacin, ofloxacin (OFLX) or the promising sparfloxacin that was as active as INH in a murine tuberculous (TB) model (6).

It has been recognized that phagocytes and their mediators play a crucial role in generating and even worsening the clinical aspects of infectious diseases . Deterioration of granulocyte function results in an impaired host defence against invading microorganisms. During infection, a reduction of various granulocyte function has been demonstrated (7,8).

The investigation of the possible immunomodulatory influence of well known antibiotics could be a new approach to the treatment of infections. Some antituberculous drugs alone or in combination could enhance on PMN functions.

A variety of methods of immunomodulation and immunotherapy have been used to improve the efficacy during the treatment of many diseases.

Today new antituberculous drugs and new combination regiments have been improved against the tuberculous resistance. The effects of these drugs on the immune system of host has been unknown yet .

The antituberculous drugs which are used in combination in order to prevent the drug resistance might effect the immune cell functions of the host positively or negatively by showing additive, antogonistic, or synergystic interaction between each other.

The aim of this study is to investigate the in vitro effects of both primary and secondary antituberculous drugs (alone and in combination) on the functions of polymorphonuclear leukocytes (PMN's).

The results of the presents investigation may help to predict the effect of the antituberculous drugs used in the therapy on the immune sistem and may bring out new approach in antituberculous therapy.

EXPERIMENTAL

Volunteers

In our study 15 healhty young volunteers were used. PMNs were isolated from these healthy young volunteers whose average age was 23 (age ranged between 19-26) . Eight of the

volunteers were female and 7 of them were male. None of the volunteers had any disease and used any drug (The healthy young volunteers were chosen under the control of a doctor).

Antituberculous Drugs

Isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol (EMB), prothionamide (PTH) and cycloserin (CYC), Para-aminosalicilic acid (PAS) were kindly supplied by Koçak Pharmaceutical Inc. (Istanbul, Turkey), streptomycin (S) by I.E. Ulagay Pharmaceutical Inc. (Istanbul, Turkey), amikacin (A) by Eczacıbası Pharmaceutical Inc. (Istanbul, Turkey), ofloxacin (OFLX), levofloxacin (LVFX) by Aventis Pharma Pharmaceutical Inc. (Istanbul, Turkey). INH (5 μg/ml), RIF (7 μg/ml), PZA (40 μg/ml), EMB (7 μg/ml), S (25 μg/ml), A (24 μg/ml), CYC (10 μg/ml), PAS (90 μg/ml) were prepared as a stock solutions at the therapeutic serum concentrations in sterile distilled water. Ofloxacin OFLX (2,9 μg/ml), LVFX (2,8 μg/ml) were prepared as a stock solutions at the therapeutic serum concentrations in Hanks's balanced salt solution (HBSS). PTH (1,6 μg/ml) were prepared as a stock solutions at the therapeutic serum concentrations in methanol. Stock solutions were diluted as necessary in HBSS.

Preparation of PMNs

Human PMNs were prepared by modification of the method of Alexander et al (9) .In the modified method Ficoll was used in place of dextran and serum opsonins, also number of neutrophils were standardized in order to determine both increases and decreases in phagocytosis and intracellular killing activity PMNs were counted by microscope instead of standart pour plate tecnique.

Briefly, whole blood of healthy volunteers in ethylenediaminetetraacetic acid (EDTA, Sigma) was centrifuged at 2500 rpm (1048 X g) for 30 min.. The buffy coat layer was removed, added to Ficoll – Hypaque plus polymorphprep (Sigma) solution and was centrifuged at 3000 rpm (1509 X g) for 30 min. The PMN layer was removed and washed three times in Hanks's balanced salt solution (HBSS) without Ca^{+2} or Mg^{+2} Finaly, cell suspension were adjusted to $1x10^6$ cells ml in HBSS. The PMNs were found to be > 99 % viable by trypan blue (0,4 % in phosphate-buffered saline, Sigma) exclusion. Blood was taken between $9.^{00}$ and $10.^{00}$ a.m. (9,10).

Phagocytosis and Intracellular Killing Activity

C. albicans was used to measure the phagocytic and intracellular killing of PMNs. Yeast was a clinical isolate (*C. albicans* 4826) obtained from the Clinical Microbiology Laboratory in Marmara University Hospital, Istanbul, Turkey. Yeast cells were counted and suspended in HBSS ($1x10^7$ cells/ml) without Ca^{+2} or Mg^{+2}. This suspension was prepared in fresh human serum (1/10) and incubated at 37 °C for 30 min. in a shaker incubator. PMNs (10^7 cells/ml) were incubated in each steril tube that contained antituberculous drugs INH (5 μg/ml), RIF (7 μg/ml), PZA (40 μg/ml), EMB (7 μg/ml), S (25 μg/ml), A (24 μg/ml), CYC (10 μg/ml), PAS (90 μg/ml), OFLX (2,9 μg/ml), LVFX (2,8 μg/ml) and PTH (1,6 μg/ml) alone and primer and secondary antituberculous drug combinations at therapeutic serum concentrations at 37 °C for 30 min shaker incubator (9,10).

After preincubation, opsonized yeast cells were added to the mixture of PMNs and antituberculous drugs. The mixture contained $5x10^6$ PMN/ml and $5x10^6$ yeast/ml. This mixture was incubated at 37°C for 30 min. At the 25^{th} minute of incubation, 1 ml of methylene blue (0,01 %, Sigma) was added to the mixture to stain the dead yeast cells at the 30^{th} minute of incubation. The phagocytic activity was determined by the percentage of PMNs that had phagocytosed yeast cells. The intracellular killing activity was determined by the percentage of PMNs that included killed yeast cells by PMNs were counted on a slide under microscope and the results were expressed as a percentage. All assays were performed in triplicate (9,10).

Statistical Analysis

The results were expressed by means of ± SD (Standart deviation).Differences between drugs were performed using Independent Samples T Test. P values less than or equal to 0.05 were considered to be statistically significant.

RESULTS

The effects of primary and secondary antituberculous drugs and their combinations on the PMN functions of healthy young volunteers were shown in Table 1 and Table 2.

Primary antituberculous drugs alone and their combinations slightly affected PMN functions (phagocytosis and intracellular killing activity) when compared to the control (drug-free) ($p>0.05$, Table 1).

A, significantly increased the phagocytic activity of PMN functions when compared with the control (drug-free) (67,13 ± 11,76 vs 57,47 ± 12,20, $p<0,05$) and PAS (66,87 ± 10,15 vs 57,47 ± 12,20, $p<0,05$). Intracellular killing activity of PMNs was significantly increased when compared with the control(drug-free) by OFLX (3,26 ± 1,22 vs 2,13 ± 1,45, $p<0,05$).

The other secondary antituberculous drugs alone and their combinations slightly affected PMN's phagocytic and intracellular killing activity.

DISCUSSION

As it is clearly seen from our investigation, primary and secondary antituberculous drugs, alone and in their combination has affected human polymorphonuclear leukocytes functions (phagocytic and intracellular killing activity) in vitro.

Some antibiotics were demonstrated to enhance various immune parameters such as, phagocytic and intracellular killing activity, B lymphocyte responsiveness and delayed hypersensitivity (11,12,13).

It is important to know the effect of antituberculous drugs on the immune system cell functions of the tuberculous patients

The antituberculous drugs which effect the immune system of patients with tuberculosis might effect their immune system in either positively or negatively .

It is reported that even in very low concentrations, the mycobacterial sulfides in the lysosomes, inhibit the phagolysosomial fusion. As a result, the bacilli in the phagocytic cell continue to multiply as a potential pathogen without beeing killed (14,15) . The studies show that tuberculosis is a insidious diseases based upon the struggle between the immune system of the host and the bacile. That is why it is important to know the possible beneficial and hazardous effects of the drugs used in the therapy of the patients with tuberculosis.

Duncker et al (16) showed that INH, RIF at concentrations higher than the therapeutic dose inhibited the phagocytic activity of the neutrophils.

RIF is easily penetreated into the cells and has bactericidal effect against it. This antibiotic inhibits protein synthesis of phagocytic cells and decreases the suspectibility and chemotaxis of

Table 1. In vitro effect of primary antituberculous drugs alone and their combinations on the PMN functions (Phagocytosis and intracellular killing activity) of healthy young volunteers (n= 15).

Drugs	Therapeutic Concentration (μg/ml)	Phagocytic activity* (%)	Intracellular killing activity* (%)
Isoniazid	0	69,73 ± 14,46	10,66 ± 3,81
	5	66,00 ± 12,94	12,06 ± 5,33
Rifampicin	0	69,73 ± 14,46	10,66 ± 3,81
	7	70,06 ± 12,94	12,60 ± 4,74
Pyrazinamide	0	73,87 ± 14,19	10,93 ± 5,13
	40	76,60 ± 09,42	12,80 ± 5,80
Ethambutol	0	73,87 ± 14,19	10,93 ± 5,13
	7	72,73 ± 12,87	13,07 ± 7,30
Streptomycin	0	73,87 ± 14,19	10,93 ± 5,13
	25	71,13 ± 15,09	12,80 ± 6,06
INH+RIF	0	73,87 ± 14,19	10,93 ± 5,13
	5+7	71,67 ± 11,86	11,93 ± 5,86
INH+RIF+PZA+EMB	0	69,73 ± 14,46	10,66 ± 3,81
	5+7+40+7	76,06 ± 10,75	12,20 ± 4,90
INH+RIF+PZA+EMB+S	0	69,73 ± 14,46	10,66 ± 3,81
	5+7+40+7+25	77,93 ± 10,78	08,26 ± 4,71

INH: isoniazid, RIF: Rifampicin, PZA: Pyrazinamide, EMB: Ethambutol, S: Streptomycin

The effects of antituberculous drugs were compared with the control values (0=drug-free) by using Independent Samples T Test and the data shown is by means of ± SD, p*>0.05.

Table 2. In vitro effect of secondary antituberculous drugs alone and their combinations on PMN functions (Phagocytosis and intracellular killing activity) of healthy young volunteers (n=15)

Drugs	Theraupeutic Concentration (μg/ml)	Phagocytic Activity (%)	Intracellular killing activity (%)
1/100 Methanol	0	57,47 ± 12,20	2,40 ± 1,72
	-	58,67 ± 12,29	2,07 ± 1,03
Prothionamide	0	57,47 ± 12,20	2,40 ± 1,72
	1,6	64,67 ±12,88	1,93 ± 1,33
Amikacin	0	57,47 ± 12,20	2,40 ± 1,72
	24	67,13 ± 11,76*	2,60 ± 1,54
Cyclocerine	0	57,47 ± 12,20	2,40 ± 1,72
	10	60,60 ± 12,80	3,13 ± 1,99
PAS	0	57,47 ± 12,20	2,40 ± 1,72
	90	66,87 ± 10,15*	3,27 ± 2,31
Ofloxacin	0	59,26 ± 14,64	2,13 ± 1,45
	2,9	67,66 ± 14,33	3,26 ± 1,22*
Levofloxacin	0	59,26 ± 14,64	2,13 ± 1,45
	2,8	62,86 ± 13,86	2,80 ± 1,78
A+ OFLX+ CYC+ PTH+ PAS	0	64,13 ±13,42	2,86 ± 2,11
	24+2,9+10+1,6+90	63,86 ± 12,50	2,53 ± 2,09
A+OFLX+CYC+PTH+PAS +PZA	0	64,13 ±13,42	2,86 ± 2,11
	24+2,9+10+1,6+90 +40	63,20 ± 13,99	3,13 ± 2,26
A+OFLX+CYC+PTH+EMB+PZA	0	64,13 ±13,42	2,86 ± 2,11
	24+2,9+10+1,6+7+ 40	65,86 ± 13,97	3,33 ± 1,98
A+ LVFX+ CYC+ PTH+ PAS	0	64,13 ±13,42	2,86 ± 2,11
	24+2,8+10+1,6+90	55,86 ± 16,08	1,86 ± 1,23

A: Amikacin, OFLX: Ofloxacin, CYC: Cycloserine, PTH: Protionamide, PAS: Para amino salicylic acid, EMB: Ethambutol, PZA: Pyrazinamide. LVFX: Levofloxacin, 0: kontrol (drug-free).

The effects of antituberculous drugs were compared with the control values (drug-free) by using Independent Samples T Test and the data shown is by means of ± SD,* $p<0.05$.

B and T lymphocytes against mitogenes, but when used at therapeuetic doses (7μg/ml) it increases CD1b expression of cytokine (12,13).

RIF impairs various PMN functions such as chemotaxis and the oxidative burst.
Studies have shown that RIF reduces humoral and cell-mediated immunity (17).

Gürer et al showed that (17) RIF at concentration of (7μg/ml) did not effect neither of the PMN functions of elderly patients and healthy young volunteers when compared with the control values. Additionally, at the same study, at concentration of 20+14μg/ml cefodizime+rifampicine combination in significantly increased PMN's phagocytic and intracellular killing activity of healthy young volunteers and elderly patients.

Okuyan et al (15) demonstrated that rifampicine at concentration (7μg/ml) did not effect neither of PMN functions of healthy young volunteers when compared with the control.

Demkow et al (18) showed that RIF at concentration of (5μg/ml) inhibited human PMN oxidative burst.

In this study RIF (7μg/ml) alone did not significantly effect phagocytic activity and intracellular killing activity of PMN's when compared with the control ($p>0.05$).

Okuyan et al (15) demonstrated in their study that INH, RIF, PZA, EMB, and S alone did not significantly affect phagocytic and intracellular killing activity of PMN's when compared with the control group. The other tuberculous drugs (PTH, A) showed increased significantly the phagocytic activity ($p<0.05$). OFLX showed increased significantly both of PMN functions when compared with the control (drug-free) values ($p<0.005$, $p<0.001$).

In the present investigation also we found that primary antituberculous drugs (INH, PZA, EMB, S) alone and their combination did not affect PMN functions (phagocytosis and intracellular killing activity) when compared with the control group ($p>0.05$) (Table 1).

Duncker et al (16) demonstrated that there was no effect with high and therapeutic concentration of ethambutol and streptomycin chemiluminescence reaction during the zymosan phagocytosis.

Zeis (19) demonstrated that PZA, S and EMB alone and their combination did not inhibit the production of antimicrobial reactive radicals by PMN's.

In a study which investigated the effects of some antibiotics on PMN functions of healthy young volunteers, it was shown that A alone did not significantly effect both PMN functions of healthy young volunteers when compared with the control. Also in the same study was shown that imipenem alone significantly enhanced effect intracellular activity of PMN's ($p<0.001$). However, when A combined with imipenem (21+50μg/ml) it was demonstrated that this combination significantly increased the PMN's phagocytic activity ($p<0.05$) and intracellular killing activity of the same group ($p<0.001$) (11).

Adalati et al (20) in their study done on patients with chronic hepatitis B demonstrated that A (8μg/ml) alone did not effect both PMN functions (phagocytosis and intracellular killing activity). However when A was combined with cytokine (8μg/ml) + IFN α-2a (10IU/ml) the combination was used both PMN functions of the same groups slightly increased, but this was not statistically significant.

Many studies demonstrated that A did not impair phagocytic and intracellular killing activity of normal human neutrophil at therapeutic concentrations in vitro (21,22,23) increased by A ($p<0.05$) in addition intracellular killing activity did not affected in vivo (24).

Although Ferrari at al (24) showed inhibition of phagocytic and intracellular killing activity of amikacin, Okuyan et al (15) reported in their study they showed that phagocytic activity of PMN's was significantly increased by amikacin ($p<0.001$) when compared control values.

In our study A and PAS are secondary drugs significantly increased the phagocytic activity, OFLX significantly increased the intracellular killing activity when compared with the control ($p<0.05$). The other primary and secondary antituberculous drugs did not significantly effect the phagocytic and intracellular killing activity when compared with that of the control values ($p>0.05$).

Kadir T (25) showed that ofloxacin at sub-MIK concentration of 1.56 μg/ml significantly increased of the human PMN oxidative burst.

Nielsen et al (26) showed that OFLX at 4xMIC of 4μg/ml significantly increased the neutrophil intracellular killing activity when compared with ciprofloxacin, lomefloxacin, flerofloxacin. Also they reported that ofloxacin and ciprofloxacin at therapeuetic concentrations increased the intracellular killing of S.aureus in neutrophil.

Azuma et al. (27) demonstrated that the innate host defence system is regulated in part by the number and activation of the function of neutrophil and macrophages. The immunological effects of variety of quinolones on host defence functions of macrophages are reported OFLX, levofloxacin LVFX and others quinolones significantly inhibited phagocytosis of E.coli by macrophages, OFLX , LFLX, TFLX and LVFX were effective in significantly increasing the production of hydrogen peroxide , while the other agents did not. These results suggest that the quinolones at a therapeutic concentration differentially affect phagocytosis, adhesion and the production of hydrogen peroxide by macrophages.

Okuyan et al (15) have shown that INH+RIF+S combination had significantly increased PMN's phagocytic activity, but did not affect PMN's intracellular killing activity.

In the study of Okuyan et al INH+RIF+PZA, INH+RIF+EMB and in our study , INH+RIF, INH+RIF+PZA+EMB, INH+RIF+PZA+EMB+S combinations insignificantly increased PMN's functions.

The addition of PZA, EMB, PZA+EMB, PZA+EMB+S combinations to the drug regiment was not as significant as the addition of S to INH+RIF combination. The same investigators (15) demonstrated that A+OFLX, A+OFLX+CYC, A+OFLX+CYC+PTH+EMB combinations significantly increased PMN's phagocytic activity, but did not affect the PMN's intracellular killing activity ($p<0.01$, $p<0.05$ respectively).

In our study A and PAS at therapeutic concentrations significantly increased PMN's phagocytic activity, OFLX significantly increased PMN's intracellular killing activity ($p<0.05$) when the drugs in table 2 were added to these combinations (A +OFLX+CYC+PTH+PAS, A+OFLX+CYC+PTH+PAS+PZA, A+OFLX+CYC+PTH+ EMB+PZA). However, A+LVFLX+CYC+PTH+ PAS combination did not significantly increase the PMN functions .

In another study (17) RIF+cefodizime, Amikasin+imipenem combination (11) significantly increased the PMN functions of healthy young volunteers and elderly patients.

Antituberculous drug combinations that used in the present study were selected according to the most common regiments in management of multi drug resistant tuberculosis in Turkey (28) and standard therapy (29) .

Both phagocytic activity and intracellular killing activity of PMNs were insignificantly effected by the antituberculous drug combinations by respectively (INH+RIF, INH+RIF+PZA+EMB, INH+RIF+PZA+EMB+S, A+OFLX+CYC+PTH+PAS, A+OFLX+ CYC+PTH+PAS+PZA, A+OFLX+CYC+PTH+EMB+PZA, A+LVFX+CYC+ PTH.

In the present investigation A and PAS alone enhanced phagocytic activity of PMNs ($p<0.05$), ofloxacin enhanced intracellular killing activity of PMNs ($p<0.05$) although in their drug combination no adverse or synergistic effect were observed.

The results show that none of the primary and secondary antituberculous drug alone or in combination significantly decreased PMN functions .

CONCLUSION

Antituberculous drugs and their combination with a stimulatory effect on PMN's function would be useful in the treatment of infections in patients with tuberculosis. When the appropriate antituberculous drugs are chosen for the treatment it will be beneficial to consider the effects of drugs on immune system cell functions, because these effects either can be not only negative or positive, but also can neutralize negative effects of each other. Immunomodulatory effects must be considered together with antituberculous activity before the prescription of antituberculous drugs.

As it is seen from our investigation the relationship between immune system, microorganism and the drug must be kept in mind during treatment. The process might brought a new immunotherapeutic approach to the therapy of patients whose immune system is deteriorated or suppressed.

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