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Original article

IN VITRO EFFECTS OF ANTIDEPRESSANT DRUGS ON POLYMORPHONUCLEAR LEUKOCYTE FUNCTIONS OF HEALTHY VOLUNTEERS

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Abstract

In our study the effects of the antidepressant drugs buspirone (0.0025µg/ml), sertraline (0.19µg/ml), citalopram (0.286µg/ml, 0.502µg/ml, 0.703µg/ml) and fluoxetine (0.015µg/ml, 0.035µg/ml, 0.055µg/ml) on polymorphonuclear leukocyte (PMN) function of 20 healthy young volunteers, whose mean age was 25 were investigated in vitro. PMNs ($1x10^7$ cell/ml) were isolated by ficoll-hypaque gradient centrifugation method from venous blood with EDTA. Phagocytosis and intracellular killing activity were assayed by modifying Alexander's method. Fluoxetine at 0.035µg/ml concentration has significantly increased the PMN's phagocytic activity, of the healthy young volunteers but did not effect their PMN's intracellular killing activity. The results might bring a new immunotherapeutic approach to the therapy of the major depressant patients whose immune system is suppressed.

Key words: *Polymorphonuclear leukocyte, Antidepressant drugs, Phagocytosis, Intracellular killing activity*

Antidepresan İlaç ve Kombinasyonlarının İnsan Polimorf Nüveli Lökosit Fonksiyonları Üzerine in Vitro Etkisi

Çalışmamızda antidepresan ilaçlardan buspiron (0.0025µg/ml), sertralin (0.19µg/ml), sitalopram (0.286µg/ml, 0.502µg/ml, 0.703µg/ml), fluoksetin (0.015µg/m, 0.035µg/ml, 0.055µg/ml)'in PNL fonksiyonları (fagositoz ve hücre içi öldürme aktivitesi) üzerine etkisi yaş ortalaması 25 olan 20 sağlıklı gönüllüde in vitro koşullarda araştırılmıştır. PNL'ler ($1x10^7$ hücre/ml) EDTA'lı venöz kandan ficoll-hypaque gradient yöntemi ile ayrılmıştır. Fagositoz ve hücre içi öldürme aktivitesi tayininde Alexander ve arkadaşlarının yöntemi değiştirilerek kullanılmıştır. Fluoksetinin 0.035µg/ml'lik dozu PNL'lerin fagositik aktivitesini anlamlı olarak artırmıştır ($p<0,05$), ancak PNL'lerin hücre içi öldürme aktivitesini etkilememiştir ($p>0.05$). Elde edilen bulgular immün sistemi zayıflamış major depresyonlu hastaların tedavisine yeni bir immünoterapik yaklaşım getirebilir.

Anahtar kelimeler: *Polimorf nüveli lökosit, Antidepresan ilaçlar, Fagositoz, Hücre içi öldürme aktivitesi*

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INTRODUCTION

Since there is biological tendency in some people for the development of depression there are various reasons that can cause depression (1). It is known that there is direct relationship between the immune system and the central nervous system by hormones and neurotransmitters. In order to access the relationship between the immune system and the mental stress together with major effects of this illness many investigations have been done. The common point at the investigations is that stress and depression have negative effects on the immune system (2,3).

Since there is an important reduction in the cytotoxicity of natural killer cells in patients with depression, there is a decrease in optimal functions of CD4 and macrophage cells, the production of cytokine production (IL-2, INF-γ), total immune response, antibody response, neutrophile phagocytosis, negative acute phase protein, reduction in the weight of the thymus and spleen (4).

Freire-Garabal *et al* (5) have determined that auditory stress has negative effects on the immune system (thymus cells, spleen cells, lymphocytes, weight of thymus, blastogenic spleen cells, phygocytosis in vitro and in vivo) of mice. The same investigators have shown that nefazodone has increased the positive effects on the test mice's immune system cell functions (thymus, spleen, lenfocytes) and peritoneal mice macrophages activities more than the placebo group mice that were exposed to stress. The nefadozone treatment significantly increased the phagocytic activity of peritoneal mice macrophages when compared with the placebo group which was exposed to stress ($p<0.01$) (5).

Freire-Garabal *et al* (6) have investigated the effects of an antidepressant drug fluoxetine (1,2,5,10,20 mg/kg) on the phagocytic activity of macrophages of the mice that were exposed to stress in vitro and in vivo. The same investigators have determined that depending on the dose fluoxetine (2 mg/kg) has significantly increased the phagocytic activity of macrophages of the mice more than placebo group which was exposed to stress ($p<0.05$) .

In other study Freire-Garabal *et al* (7) have investigated the therapeutic dose of buspirone (0.5, 1, 2 mg/kg/daily) on the phagocytic activity and cytotoxic activity of NK (Natural Killer) cells of peritoneal macrophages of mice which was exposed to stress (1-16 days). The investigators have declared that in stressed mice when buspirone is given intraperitoneally (0.5-1mg/kg/daily) it increased the phagocytic activity of the peritoneal macrophages (in vitro and in vivo) and the cytotoxic activity of the NK cells depending on the dose.

Ying *et al* (8) have investigated the effects of the clomipramine (40μmol/L), imipramine (100μmol/L) and citalopram (100μmol/L) on monocytes in vitro for 11 days. It was shown that human monocytes treated with clomipramine, imipramine and citalopram never developed the morphology characteristic of macrophage-like cells, decreased phagocytic cells and had little influence the expression of CD14, CD16 and HLA-DR .

In our investigation we have aimed to study the effects of the antidepressant drugs which are generally used in treatment of patients with depression on PMN's phagocytic and intracellular killing activity of the healthy volunteers. In future studies, we aim to compare the effects of the same antidepressant drugs at the same dose on PMN functions of patients with depression and those of healthy volunteers in vitro and in vivo, also to determine the positive and negative effects of the antidepressant drugs which are generally used in treatment patients with depression on immune system cells.

EXPERIMENTAL

Volunteers

In our study 20 healhty young volunteers were enrolled. Peripheral blood polymorphonuclear cells (PMNs) were isolated from these healthy young volunteers whose age range was 20-30. None of them had any disease and used any drug (The healthy young volunteers were chosen under the control of a doctor).

Antidepressant Drugs

The drugs used in the study and their therapeutic concentration: Buspirone (0.0025 μg/ml), sertraline (0.19μg/ml), citalopram (0.286μg/ml, 0.502 μg/ml, 0.703μg/ml), and fluoxetine (0.015μg/ml, 0.035 μg/ml, 0.055μg/ml). Buspirone was prepared as a stock solution at the therapeutic serum concentration in absolute ethanol. Sertraline, fluoxetine and citalopram were prepared as stock solutions at the therapeutic serum concentrations in methanol.

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 PMNs were counted by microscope instead of standart pour plate tecnique.

The PMNs were isolated from the venous blood by Ficoll – Hypaque gradient centrifugation method.

Briefly, whole blood of healthy volunteers in ethylenediaminetetraacetic acid (EDTA, Sigma) was centrifuged at 2500 rpm (1048g) for 30 min. The buffy coat layer was removed, added to Ficoll – Hypaque plus polymorphprep (Sigma) solution and was centrifuged at 3000 rpm for 30 min. The PMN layer was removed and washed three times in Hanks's balanced salt solution (HBSS). Finally, cell suspension was adjusted to 1x107 cells ml in HBSS (9-15).

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 (1x107 cells/ml). This suspension was prepared in fresh human serum (1/10) and incubated at 37 oC for 30 minutes in a shaker incubator for opsonization. PMNs (107 cells/ml) were incubated in each steril tube that contained antipressant drugs buspirone (0.0025 μg/ml), sertraline (0.19μg/ml), citalopram (0.286μg/ml, 0.502 μg/ml, 0.703μg/ml), and fluoxetine (0.015μg/ml, 0.035 μg/ml, 0.055μg/ml) at different therapeutic serum concentrations at 37 oC for 30 min shaker incubator (9-15).

After preincubation, opsonized yeast cells were added to the mixture of PMNs and antidepressant drugs. The mixture contained 5x106 PMN/ml and 5x106 yeast/ml. This mixture was incubated at 37oC for 30 min. At the 25th minute of incubation, 1 ml of methylene blue (0.01 %, Sigma) was added to the mixture to stain the dead yeast cells . The phagocytic activity was determined by counting PMNs that had phagocytosed yeast cells. The intracellular killing activity was determined by counting PMNs that included killed yeast cells on a slide under microscope and the results were expressed as a percentage. All assays were performed in triplicate (9-15).

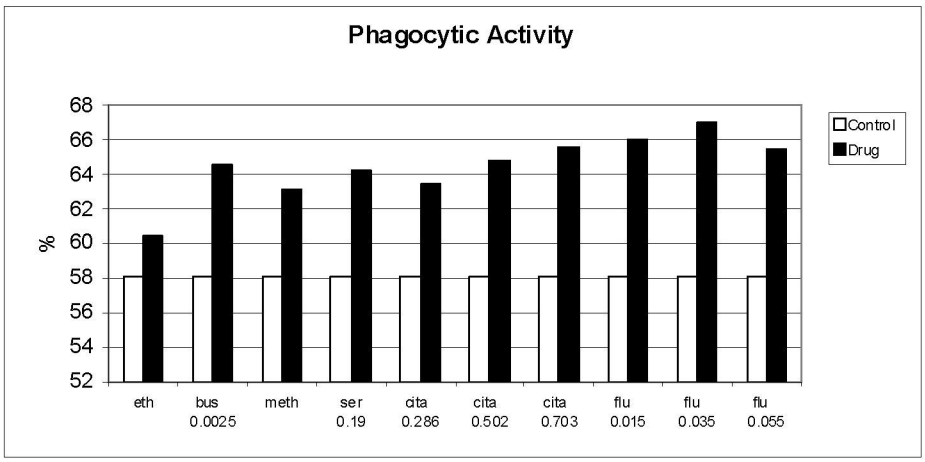
Statistics

The results were expressed by means of ± SD (Standart deviation). Statistical analyses were performed using repeated measures of ANOVA Test. P values less than or equal to 0.05 were considered to be statistically significant.

RESULTS

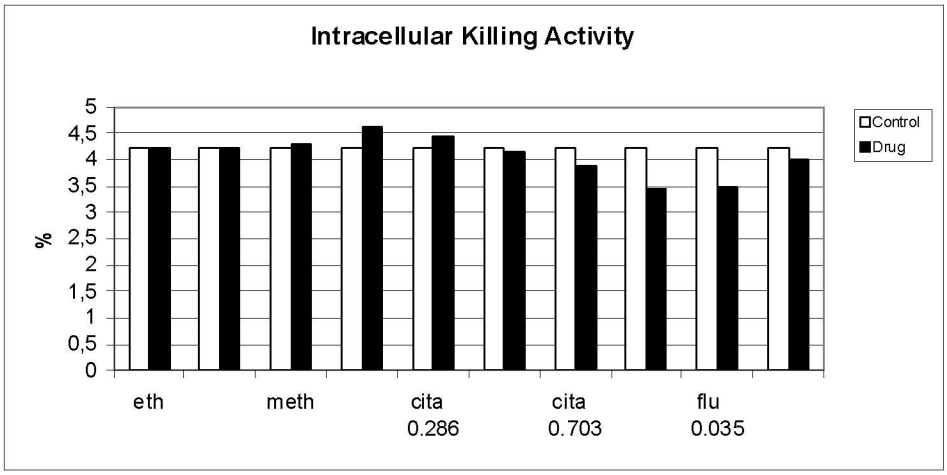
As it is seen from Figure 1 fluoxetine (0.035 μg/ml) significantly increased the phagocytic activity of healthy young volunteers' PMNs ($p<0.05$). However; the same did not significantly affect the PMN's intracellular killing activity of healthy young volunteers' PMNs.

While all of the therapeutic doses of other antidepressant drugs increased the PMN's phagocytic activity insignificantly, they do not affect their intracellular killing activities ($p>0.05$) (Figure 1,2 and Table 1).



Eth: Ethanol, Bus: Buspirone, Met: Methanol, Ser: Sertraline, Sito: Citalopram, Flu: Fluoxetine, 0: Control (Drug-free)

Figure 1. The effect of antidepressant drugs on the PMN's phagocytic activity of the healthy young volunteers.



Eth: Ethanol, Bus: Buspirone, Met: Methanol, Ser: Sertraline, Sito: Citalopram, Flu: Fluoxetine, 0: Control (Drug-free)

Figure 2. The effect of antidepressant drugs on the PMN's intracellular killing activity of the healthy young volunteers

Table 1. The effect of antidepressant drugs on the PMN functions (Phagocytosis and intracellular killing activity of the healthy young volunteers (n=20).

	Therapeutic Concentration(µg/ml)	Phagocytic Activity (%)	Intracellular killing activity (%)
1/1000 Ethanol	0	58.05 ±10.86	4.20 ± 2.65
	-	60.50 ± 11.29	4.20 ± 3.10
Buspirone	0	58.05 ± 10.86	4.20 ± 2.65
	0.0025	64.55 ± 9.61	4.60 ± 3.56
1/100 Methanol	0	58.05 ±10.86	4.20 ± 2.65
	-	63.10 ± 8.40	4.30 ± 3.67
Sertraline	0	58.05 ±10.86	4.20 ± 2.65
	0.19	64.25 ± 10.21	4.85 ± 3.77
Citalopram	0	58.05 ±10.86	4,20 ± 2.65
	0.286	63.45 ± 11.77	4.45 ±3.20
Citalopram	0	58.05 ±10.86	4.20 ± 2.65
	0.502	64.80± 10.68	4.15 ± 3.38
Citalopram	0	58.05 ±10.86	4.20 ± 2.65
	0.703	65.55 ± 10.58	3.90 ± 2.24
Fluoxetine	0	58.05 ±10.86	4.20 ± 2.65
	0.015	65.95 ± 12.63	3.45 ±3.12
Fluoxetine	0	58.05 ± 10.86	4.20 ± 2.65
	0.035	66.95 ± 9.52*	4.25 ± 3.84
Fluoxetine	0	58.05 ± 10.86	4.20 ± 2.65
	0.055	65.45 ± 10.33	4.00 ± 2.97

The effects of antidepressant drugs were compared with the control values (drug-free) by using Repeated Measures of Anova and the data shown is by means of ± SD, * $p < 0.05$.

DISCUSSION

Any supression on the activities of PMN, monocyte, macrophage and lymphocyte functions which are important components of the host defence system might deteriorate the control of the immune system of patients major depressed patients.

It is declared that most of the drugs used today (for instance; antineoplastic agents, analgesics, antibiotics, antifungals, antidepressants drugs) have affected the functions of the immune system cells and cytokines (11).

In searching the direct effects of the antidepressant drugs, human and animal immune system cells and under in vitro and in vivo conditions are used. However, the number of in vivo human experiments are few.

Investigations on the relationship between depression, cytokines, immune system cell functions and antidepressant drugs have recently started.

On the other hand, in the investigations that have been with the depressed patients it is declared that proinflamatuar cytokines (IL-1, IL-6 and TNF β) and acute phase proteins that are the indicators of the efficiency of and immune cells have increased and besides that it has been said that there have changed in the other immune system functions (2).

In our study the effects of one therapeutic dose of buspirone (0.0025 μg/ml) and sertraline (0.19 μg/ml) which are important drugs used in the therapy of patients with depression, and also three different therapeutic doses of citalopram and fluoxetine (0.286 μg/ml, 0.502 μg/ml, 0.703 μg/ml, 0.015 μg/ml, 0.035 μg/ml, 0.055 μg/ml, respectively) on the healthy volunteers' PMN functions (phagocytic activity intracellular killing activity) have been investigated.

Our findings show that the therapeutic dose of buspirone (0.0025μg/ml) has insignificantly increased the phagocytic activity of healthy human PMNs, but it has not changed the intracellular killing activity of PMNs when compared with control PMNs's intracellular killing activity. In a study Freire-Garabal et al (7) have investigated the effects of buspirone on the immune system of mice exposed to a chronic audotory stressor. The investigators found that daily injection of 0.5 and 1mg/kg (intraperitoneal) dose of buspirone resulted in dose-dependent reduction in the stress-induced suppression of the NK cell activity and the in vitro and in vivo activity of phagocytosis.

In our study sertraline (0.19μg/ml) has insignificantly increased the intracellular killing activity and phagocytic activity of the healthy human PMNs when compared with control PMNs.

Leonard and Song (16) have shown that long-term sertraline treatment has increased the percentage of neutrophile and the proliferation of T lymphocytes. Their results supported our findings.

In our study the three different therapeutic doses of citalopram (0.286, 0.502, 0.703μg/ml) have insignificantly increased the phagocytic activity of PMNs. Citalopram (0.286μg/ml) has insignificantly increased intracellular killing activity. The other therapeutic doses of citalopram (0.502 μg/ml, 0.703 μg/ml) did not affect PMN's intracellular killing activity.

Kubera et al (17) has investigated the immunological effects of citalopram (10mg/kg/i.p) on the C-57B1 mice. They have shown that the citalopram treatment increased the proliferative activity of the spleen cells, 1 week treatment significantly increased IL-2 and IL-6 ($p<0.05$) and also that it significantly decreased IL-4. In the second week the increase of IL-6 was insignifficant. However, IL-10 has increased insignificantly. It is stated that during 4-week citalopram treatment insignificantly decreased IL-2. Other immunologic values except IL-4 was going on and there was no difference in IFN-α values. However, in the same investigation 28-day treatment there was an important decrease in the metabolic activity of spleen cells($p<0.01$).

In our study the three therapeutic concentration of citalopram had not affected healthy volunteer's PMN functions.

Therapeutic doses of fluoxetine (0.015 μg/ml, 0.055 μg/ml) has increased the phagocytic activity of PMNs insignificantly when compared to the control and also 0.035 μg/ml therapeutic dose of fluoxetine has increased the phagocytic activity of PMNs significantly ($p<0.05$). However; the same three therapeutic doses of fluoxetine did not change the intracellular killing activity significantly.

Freire-Garabal et al (6) have stated that the phagocytic activity of peritoneal macrophages in control mice that were exposed to stress at in vitro and in vivo conditions (2,4,6,8 and 16 days) significantly decreased when compared with the control. The same investigators have applied various doses of fluoxetine (2-20mg/kg) on stressed mice. It was found that 5mg/kg fluoxetine significantly increased the phagocytic activity of peritoneal macrophages in mice and it significantly decreased the level of serum ACTH ($p<0.01$). They have found that 5mg/kg

dose of fluoxetine did not change the phagocytic activity of macrophages and the serum ACTH level in mice that were not exposed to stress.

Kubera et al (17) have shown that chronic fluoxetine therapy (4 weeks) inhibits prostate carcinoma and spontaneous neoplasm in rodents and they have declared that it significantly increases IL-2, IL-6 and IL-10,but it significantly decreases IL-4.

The studies show that antidepressant drugs affect the natural and cellular immune mechanism of the immune system positively depending on the dose during the acute and chronic treatment. The results of our study support these point of view.

The therapeutic doses of the antidepressant drugs that were used in our study (buspirone 0.0025μg/ml, sertraline 0.19μg/ml, citalopram 0.286μg/ml,0.502μg/ml, 0.703μg/ml, fluoxetine 0.015μg/ml,0.055μg/ml) have insignificantly increased the PMN's phagocytic activities of healthy volunteers. The same doses of the antibiotics did not affect PMN's intracellular killing activity.

Fluoxetine (0,035μg/ml) has significantly increased PMN's phagocytic activity of healthy volunteers ($p<0,05$). It did not change PMNs'intracellular killing activity of healthy volunteers.

CONCLUSION

By choosing the proper antidepressant drug and it's dose during treatment, a new immunotherapic approach can be brought to the treatment of the patients with weak immune system and in our opinion that beneficial results of the treatment could be obtained soonly.

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