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IN VITRO IMMUNOREGULATORY EFFECTS OF ANTIDEPRESSANTS IN HEALTHY VOLUNTEERS

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In vitro immunoregulatory effects of antidepressants in healthy volunteers. A. SZUSTER-CIESIELSKA, A. TUSTANOWSKA-STACHURA, M. SŁOTWIŃSKA, H. MARMUROWSKA-MICHAŁOWSKA, M. KANDEFER-SZERSZEŃ. Pol. J. Pharmacol., 2003, 55, 353–362.

Major depression is accompanied by an activation of the inflammatory response system (IRS) and antidepressants may have immunoregulatory activities.

This study was carried out to compare the effect of imipramine, mianserin and lithium on the *in vitro* production of Th1-like cytokines, such as IL-2, IFN-γ, lymphotoxin and Th2-like cytokines such as IL-4, IL-10 as well as IL-12 and TGF-β. Peripheral blood mononuclear cells (PBMC) of 16 healthy volunteers were stimulated with polyclonal activators (phytohemagglutinin with lipopolysaccharide PHA + LPS) with or without incubation with imipramine, mianserin (1 μM) or lithium (1 mM). Imipramine and mianserin exhibited similar activities enhancing unstimulated IFN-γ and IL-10 production. In PHA + LPS-stimulated PBMC both antidepressants inhibited IFN-γ, IL-2 and lymphotoxin production (Th1-like cytokines) as well as IL-12 and IL-4 production. Under the same *in vitro* conditions, both antidepressants stimulated production of negative immunoregulatory cytokines such as IL-10 and TGF-β. Lithium differed significantly from imipramine and mianserin, as it enhanced IL-2, IFN-γ, IL-10 and TGF-β production and inhibited only IL-4. All three examined antidepressants reduced IFN-γ/IL-10 ratio. None of the antidepressants at the used concentrations induced apoptosis in PBMC so those changes in cytokine production were not the result of selective killing of certain cell subpopulations. Imipramine and mianserin at high concentrations negatively influenced reactive oxygen species (ROS) production in neutrophils, however, at concentrations in the therapeutical range none of the antidepressants used influenced "oxidative burst" in neutrophils. The results indicate that antidepressants exert immunoregulatory effects on human leukocyte functions, especially on cytokine production.

Key words: *antidepressants, imipramine, mianserin, lithium, peripheral blood leukocytes, cytokines, reactive oxygen species*

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INTRODUCTION

Major depression is accompanied by an activation of the inflammatory response system (IRS). Patients with major depression show an increased number of peripheral blood leukocytes and increased CD4+/CD8+ ratio, increased plasma concentrations of positive acute phase (AP) proteins, such as haptoglobin, C-reactive protein and α1-antitrypsin and an increased serum level of some proinflammatory cytokines and their receptors such as IL-6, IL-6R, IL-2, IL-2R and IL-1β, but decreased production of IFN-α [9, 20, 22, 26, 40, 45].

One of major advances in the understanding of the modulation of cell-mediated immunity and the IRS was the identification of two mutually exclusive populations of Th (T helper) cells: Th1 which produce IFN-γ and IL-2 and Th2 which produce IL-4 and IL-10 which potently suppress Th1 cytokine production [6]. Th1/Th2 cytokine response is regulated by other cytokines produced mainly by monocytes/macrophages such as IFN-α and IL-12 which enhance Th1 response [31, 32]. It has been suggested that the increased production of the proinflammatory cytokines, especially IL-1β and IL-6, and the imbalance between Th1/Th2 response may play a role in the pathophysiology of depression [49].

IL-1, IL-2 and IFNs given to humans may produce behavioral alterations and symptoms similar to those observed in depression, such as anhedonia, anorexia, social withdrawal, sleep disturbances [43, 48]. Moreover, lipopolysaccharide (LPS) administration provokes depressive-like behavioral symptoms in the rats. Since LPS is known to induce several proinflammatory cytokines, such as IL-1, IL-6, TNF-α, IFN-α, depressive-like symptoms are connected with cytokine production [38].

There is some evidence that the activation of IRS, increase in monoamine catabolism, and abnormalities in lipid compounds may cause overproduction of reactive oxygen species (ROS) and, in turn, antioxidative enzyme (AOE) activities and lipid peroxidation. These phenomena may also be related to pathophysiology of major depression [2, 8, 21, 24].

Tricyclic antidepressants (TCAs) have been shown to suppress both synthesis of proinflammatory cytokines and depressive-like symptoms [38]. If increased production of proinflammatory cytokines is involved in the ethiology of depression, one would expect that antidepressive treatment has negative immunoregulatory effects. Such effect of some antidepressants has been described. In depressed patients, the treatment with antidepressants may normalize the initial increase in the number of blood leukocytes and neutrophils and in initially elevated serum IL-6 level and attenuate AP response in depressed patients [26, 39]. Moreover, antidepressants clomipramine and imipramine, significantly suppress the secretion of IL-1β, TNF-α, IFN-γ and IL-2 by leukocytes *in vitro*. On the other hand, antidepressants may enhance the production of IL-10 in whole blood cultures of normal humans, and in consequence they decrease IFN-γ/IL-10 ratio. Therefore, it has been suggested that antidepressants exert some of their antidepressive effects through their negative immunoregulatory effects [26, 47].

Lithium, which is used in the treatment of unipolar and bipolar depression and augments the clinical efficacy of TCAs and selective serotonin reuptake inhibitors (SSRI) in the treatment of depression, was also described to exert significant immunoregulatory effects. It increases the production of both proinflammatory cytokines (IFN-γ, TNF-α and IL-8) as well as anti-inflammatory IL-10 and IL-1RA [25].

Some antidepressants, such as imipramine and chlorpromazine, may inhibit lipid peroxidation in the rat brain and suppress the ability of activated macrophages to produce ROS during phagocytosis [11, 28]. On the other hand, lithium has been shown to modulate unstimulated and FMLP (fMet-Leu-Phe)-stimulated ROS production in polymorphonuclear cells of healthy volunteers at different age [10].

The aims of the present study were to compare the influence of TCA imipramine, heterocyclic antidepressant (HCA) mianserin and lithium on the production of cytokines in peripheral blood mononuclear cells (PBMC) isolated from blood of healthy volunteers. The cytokines which are known to be the product of Th1 lymphocytes such as IL-2, IFN-γ, lymphotoxin or those originating from Th2 lymphocytes such as IL-4 and IL-10, and cytokines which are mainly the product of monocytes/macrophages, and B lymphocytes such as IL-12 and TGF-β were examined. Moreover, the influence of three antidepressants on the level of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) released by neutrophils spontaneously (without induction) and after phorbol ester (PMA) induction was examined.

MATERIALS and METHODS

Subjects

Blood samples were collected from 16 healthy volunteers. There were 8 females and 8 males aged 34.2 ± 8.1. All subjects gave written informed consent. None of the subjects had a history of psychiatric disorder. None reported a history of using antidepressants or neuroleptic medications or had used benzodiazepines during the previous 3 months. All the subjects had normal values of blood parameters such as hematological measures, blood renal tests (urea and creatinine) and normal liver tests (SGOT, SGPT, γGT levels). All the subjects have been free of acute infections or allergic reactions for at least 3 weeks prior to the study. No one was addicted to alcohol. The subjects abstained from caffeine, alcohol and nicotine at least 12 h before blood sampling.

Isolation of neutrophils and PBMC

After an overnight fast, blood samples were taken in the morning (7.00–9.00 a.m.) into tubes with heparin (heparinum, Polfa, 20 U/ml). Granulocytes were separated according to the Sigma procedure: a gradient was formed by layering an equal volume of Histopaque-1077 over Histopaque-1119 (Sigma, St. Louis, USA).The whole blood was carefully layered onto the upper Histopaque-1077. The tubes were then centrifuged at 700 × g for 30 min. Granulocytes were isolated from the Histopaque-1077/1119 inter-phase and washed three times with HBSS (Hanks' Balanced Salt Solution), centrifuged (350 × g for 15 min) and suspended in HBSS. Granulocytes represented 97–98% of the isolated cells, as estimated after May-Grünwald-Giemsa staining. PBMC were also separated by density gradient centrifugation from plasma-Histopaque-1077 inter-phase, washed two times with Eagle's Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS) and suspended in the medium at 2×10^6 cells/ml. The viability of cells determined by trypan blue exclusion test was above 98%. Mononuclear cells (lymphocytes and monocytes) represented 96% of the cells (determined after May-Grünwald-Giemsa staining).

Cytokine induction

PBMC suspension was distributed into 24-well plastic plates (Falcon, Bedfors, MA, USA) at 2×10^6 cells/well and stimulated or not (spontaneous release) with the mixture consisting of PHA (Sigma) at a final concentration of 5 μg/ml and LPS (from *E. coli* 0111:B4, Sigma), at a final concentration of 25 μg/ml. At the time of stimulation with polyclonal activators, antidepressants dissolved in MEM, imipramine at a final concentration of 1 μM, mianserin (1 μM) or lithium carbonate (1 mM) were added (10 μl volume) to the PBMC in wells. MEM alone served as a respective control. Samples were incubated for 72 h in a humidified atmosphere containing 5% CO_2, at 37°C. After incubation, supernatants were collected, centrifuged and frozen immediately at –20°C and kept for no longer than 3 weeks before cytokine titration.

Cytokine measurements

Cytokines were quantified in duplicate by means of the ELISA method using commercially available kits. IL-2, IFN-γ, IL-10, IL-4, IL-12 were detected using kits from Endogen, (Woburn, MA, USA) and TNF-β or TGF-β with kits from Quantikine, R&D System, (Minneapolis, MN, USA). Each kit employed a specific monoclonal antibody immobilized on a 96 well microtiter plate that bound one of these cytokines in the aliquot and a second enzyme-conjugated specific polyclonal antibody. Following several washings in order to remove unbound substances and antibodies, a substrate solution was added to the wells. Color development was stopped by sulfuric acid and the intensity of the color was measured by a microtiter plate reader (E-max, Molecular Devices Co, Menlo Park, CA, USA) at 450 nm (correction at 550 nm or 540 nm). All samples for each cytokine were assayed at the same time in a single run with a single lot number of reagents. The detection limits were IL-2 > 2 pg/ml, IFN-γ > 2 pg/ml, TNF-β > 7 pg/ml, IL-10 > 3 pg/ml, IL-4 > 2 pg/ml, TGF-β > 7 pg/ml and IL-12 > 3 pg/ml. Intra-assay variations were less than 5%.

Apoptosis measurements

PBMC suspension was incubated with antidepressants (1 μM imipramine and mianserin or 1 mM lithium carbonate) for 24 h, centrifuged (350 × g for 10 min) and resuspended in MEM. Cell suspension (1×10^5) in 25 μl of the medium was incubated for 3 min at room temperature with 1 μl of a solution of orange acridine (100 μg/ml) with ethidium bromide (100 μg/ml) in phosphate buffered

saline (PBS). The suspension was placed on a microscopic slide and covered with a coverslip. The cells were examined with a fluorescence microscope (Olympus, Japan), scored and categorized into normal (green fluorescence), apoptotic (green with fragmented chromatin and apoptotic bodies formed) and necrotic (red stained nucleus). A total of 500 cells were examined and the percent of apoptotic, necrotic and normal cells was calculated.

Apoptosis of cells induced by antidepressants was also measured by cytochrome C release from mitochondria by using cytochrome C ELISA kit from Bender Med System (MedSystem Diagnostic GmbH, Vienna, Austria) according to the manufacturer's instruction. PBMC, after treatment with antidepressants, were centrifuged, washed in PBS, resuspended in Lysis Buffer and incubated for 1 h at room temperature. After centrifugation at 1000 × g for 15 min the concentration of cytochrome C was measured in supernatants. Briefly, samples of supernatant were transferred into wells coated with the monoclonal antibody specific for cytochrome C, washed and incubated with biotinylated second antibody. After the incubation and washing, streptavidine-HRP complex was added. After washing, a substrate solution was added to the wells. Color development was stopped by sulfuric acid and the intensity of color was measured at 450 nm (correction at 610 nm). The limit of cytochrome C detection was 0.08 ng/ml.

Measurement of O_2^- production by cytochrome C reduction assay [27]

HBSS (177.5 μl), 12.5 μl of cytochrome C solution in HBSS (a final concentration was 75 μM), 5 μl of either SOD solution (at a final concentration of 60 U/ml) or 5 μl of distilled water, and 50 μl of neutrophils suspension (a final density of 2.5×10^5 cells/well) were added into each well on 96-well plate. After 3 min of incubation, the neutrophils were treated or not with antidepressants (at a final 1 μM concentration of imipramine and mianserin or 1 mM lithium carbonate in 5 μl). The microplate was incubated at 37°C for 60 min and transferred to the microplate reader. The absorbance values at 550 nm (the differences in OD between samples with and without SOD) were converted to nanomoles of O_2^- based on the extinction coefficient of cytochrome C: $\Delta E_{550} = 21 \times 10^3\ M^{-1}cm^{-1}$. The results were expressed as nanomoles of O_2^- per 1×10^6 cells per 60 min. In some experiments, neutrophils were preincubated with antidepressants for 24 h and after that they were activated with PMA (a final concentration of 1 μg/ml), and after 60 min of incubation, absorbance at 550 nm was read in a microplate reader.

Measurement of H_2O_2 production [33]

The assay was based on horseradish-peroxidase-dependent peroxidation (HRPO) of phenol red by H_2O_2 leading to the formation of a compound that exhibits absorbance at 600 nm. Neutrophils suspension (4×10^6 cell/ml of HBSS) was distributed into wells (50 μl/well) on a 96-well microplate. Cells were covered with 50 μl/well of assay solution. The assay solution was prepared on the day of the experiment and consisted of HBSS, phenol red (Sigma, a final concentration 0.56 mM), HRPO (Serva, a final concentration 20 U/ml) and 1 μM concentration of antidepressants. In some experiments, except for antidepressants, the assay solution also contained PMA (Sigma, a final concentration of 1 μg/ml). An assay solution without PMA or without antidepressants was added to control wells. The plate was incubated at 37°C for 60 min and then the reaction was stopped by adding 10 μl/well of 1 M NaOH. After 3 min incubation, the plates were read at 600 nm in the microplate reader. The results were expressed as nanomoles of H_2O_2 per 10^6 cells per 60 min based on the phenol red extinction coefficient ($\Delta E_{600} = 19.8 \times 10^3\ M^{-1}cm^{-1}$).

Statistics

The values of cytokine concentrations which were produced under the influence of imipramine, mianserin and lithium or were modulated by antidepressants in PBMC stimulated with PHA + LPS were calculated as means ± SD. The differences in the cytokine concentrations between PBMC treated with antidepressants and respective controls were analyzed with Wilcoxon test using Statistica software. All results were considered significant at $p < 0.05$. The relations between variables were assessed by Spearman's rank order correlation coefficient.

RESULTS

Table 1 shows the effects of TCA imipramine, HCA mianserin and lithium used at therapeutic concentrations on cytokine production in PBMC

Table 1. The influence of antidepressants on unstimulated (UN) and PHA + LPS-stimulated (ST) cytokine production by peripheral blood mononuclear cells (PBMC) of healthy volunteers

Antidepressant	Conditions	Cytokines pg/ml							IFN-γ / IL-10 ratio
		IL-2	IFN-γ	Lymphotoxin	IL-12	IL-4	IL-10	TGF-β	
Control	UN	4.2 ± 0.7	2.5 ± 0.7	0	0	0	8.2 ± 0.8	229 ± 22	0.3
	ST	416 ± 44	1481 ± 108	5155 ± 912	73.6 ± 31.8	17.3 ± 6.1	1371 ± 283	1812 ± 993	1.09
Imipramine	UN	4.3 ± 0.8	50.5 ± 11.7*	0	0	0	39.8 ± 17.2*	254.4 ± 78.7	1.26
1 μM	ST	134 ± 188*	1014 ± 547*	1057 ± 832*	42.1 ± 8.8*	5.49 ± 3.9*	2232 ± 764*	2636 ± 1222*	0.45
Mianserin	UN	3.6 ± 0.7	16.5 ± 3.4*	0	0	0	17.4 ± 3.5*	220.8 ± 39.2	0.94
1 μM	ST	145 ± 203*	1136 ± 411*	1767 ± 957*	36.8 ± 21.4*	3.0 ± 2.8*	1746 ± 424*	2364 ± 1076*	0.65
Lithium	UN	4.6 ± 1.2	4.9 ± 2.0*	0	0	0	18.9 ± 0.5*	219 ± 40	0.26
1 mM	ST	501 ± 165*	1712 ± 160*	4451 ± 1266	55.2 ± 33.2	1.4 ± 0.5*	2927 ± 485*	2549 ± 1130*	0.54

* statistically significant difference in comparison to respective control at $p < 0.05$

isolated from blood of healthy subjects. Statistical analysis showed that in unstimulated PBMC cultures, the release of IFN-γ and IL-10 was significantly enhanced by the three used antidepressants. The spontaneous release of other cytokines was not affected significantly by antidepressants.

When PBMC were stimulated *in vitro* with PHA + LPS and treated with antidepressants, the effect of imipramine and mianserin differed from that of lithium. Imipramine and mianserin significantly inhibited the production of cytokines which are the product of Th1 lymphocytes, i.e. such cytokines as IL-2, IFN-γ, lymphotoxin, and also IL-12 which is mainly the product of antigen presenting cells, especially monocytes/macrophages, and is known to positively influence the production of Th1-like cytokines. The effects of imipramine and mianserin on Th2-like cytokine production were diverse. Both antidepressants enhanced the stimulated production of inhibitory IL-10 but significantly inhibited the production of IL-4. Moreover, both antidepressants also stimulated the production of other inhibitory cytokines such as TGF-β, which is known to inhibit several immune reactions.

Quite a different effect was seen when lithium was added to PHA + LPS-stimulated PBMC of healthy subjects. Lithium significantly enhanced the production of IL-2, IFN-γ (Th1 cytokines), elevated IL-10 (Th2 cytokine) and also TGF-β production, but inhibited IL-4 production. Despite the differences in the influence on cytokine production between imipramine and mianserin in comparison to lithium, all three antidepressants decreased IFN-γ/IL-10 ratio in stimulated PMBC cultures.

Table 2. Spearman's analysis of correlations between cytokines produced in PBMC of healthy volunteers stimulated *in vitro* with PHA + LPS

PBMC treated with	Cytokines	r	p
Control	IL-2/IL-10	–0.62	0.0008
	IL-12/lymphotoxin	0.57	0.01
Imipramine	IFN-γ/TGF-β	–0.33	0.01
	IL-10/ TGF-β	0.93	0.00001
	Lymphotoxin/ TGF-β	–0.69	0.003
	IL-10/lymphotoxin	–0.69	0.01
Mianserin	IL-2/IL-10	–0.51	0.03
	IL-2/lymphotoxin	0.53	0.03
	Lymphotoxin/ TGF-β	–0.64	0.006
	IL-12/IL-10	–0.51	0.04
Lithium	IL-10/ TGF-β	0.62	0.009
	Lymphotoxin/ TGF-β	–0.5	0.05
	IL-12/IL-2	0.91	0.000001
	IL-12/IL-10	–0.51	0.04
	IL-12/ TGF-β	–0.67	0.004

Statistically significant positive and negative correlations between cytokines produced in nonstimulated and PHA + LPS-stimulated PBMC are shown in Table 2. Positive correlations were observed mainly among cytokines produced by Th1 or cytokines produced by Th2 lymphocyte populations as well as between Th1-like cytokines and IL-12 or

Table 3. Induction of apoptosis and necrosis by antidepressants in PBMC of healthy subjects

Antidepressant	% of apoptotic cells	% of necrotic cells	Cytochrome C ng/ml
Control	1.4 ± 0.7	2.7 ± 1.1	12.0 ± 1.3
Imipramine 1 μM	3.4 ± 2.6	5.5 ± 1.4	11.6 ± 2.1
Mianserin 1 μM	2.9 ± 2.8	3.6 ± 2.1	11.7 ± 1.8
Lithium 1 mM	2.9 ± 3.2	3.6 ± 1.8	9.8 ± 2.4

Table 4. The influence of antidepressants on unstimulated and PMA-stimulated O_2^- and H_2O_2 production by neutrophils isolated from blood of healthy subjects

Antidepressant	Dose μM	O_2^- nmol/10^6 of neutrophils/h		H_2O_2 nmol/10^6 of neutrophils/h
		unstimulated	PMA-stimulated	unstimulated
Control	–	2.0 ± 0.8	12.9 ± 2.4	5.6 ± 2.1
Imipramine	100	0.5 ± 0.4*	9.9 ± 1.0*	5.7 ± 2.9
	1	1.8 ± 0.9	12.5 ± 0.6	6.1 ± 3.3
Mianserin	100	0.8 ± 0.7*	8.7 ± 0.6*	5.3 ± 2.7
	1	2.1 ± 1.5	11.1 ± 1.2	6.3 ± 2.2
Lithium	1000	2.4 ± 0.9	12.9 ± 0.6	6.1 ± 2.5
	100	2.9 ± 1.5	15.2 ± 1.4	6.5 ± 2.5

* statistically significant difference in comparison to respective control at $p < 0.05$

IL-10 and TGF-β but there were also negative correlations between cytokines produced by Th1 and Th2 lymphocyte populations or between IL-12 and TGF-β.

In order to examine if antidepressants may decrease Th1-like cytokine production by selective killing of Th1 lymphocyte subpopulation we analyzed apoptotic and necrotic death of PBMC caused by antidepressants using two different methods. Table 3 shows the results of the experiment in which cells after treatment with antidepressants used at therapeutic concentrations were stained with orange acridine with ethidium bromide. None of the used antidepressants induced the apoptotic or necrotic death of cells and the number of apoptotic, necrotic and normal cells was comparable to control not treated with antidepressants. In the second method, we examined cytochrome C release from mitochondria, which phenomenon is a very early event in apoptotic death of cells. None of antidepressants used at doses which were shown to influence significantly cytokine production, influenced cytochrome C release.

When the ROS production by blood neutrophils was examined, we detected that imipramine and mianserin used at high 100 μM concentration inhibited unstimulated superoxide anion production. Lithium at the used concentrations (1 mM and 100 μM) did not significantly influence superoxide release (Tab. 4). None of the used antidepressants influenced unstimulated H_2O_2 production. When neutrophils were stimulated *in vitro* with PMA, their "oxidative burst" was significantly inhibited by 100 μM mianserin and imipramine. Lower concentrations of both antidepressants were not active. Lithium did not exert any influence on superoxide anion production.

DISCUSSION

The main findings of this study were that monoamine reuptake inhibitory antidepressant such as imipramine and autoreceptor α2 inhibitor and enhancer of noradrenaline release, mianserin exerted a significant effect on cytokine production. Both antidepressants enhanced the unstimulated production of IFN-γ and IL-10. When PBMC were stimulated with polyclonal activators (PHA + LPS), antidepressants had a suppressive effect on proinflammatory/stimulatory cytokines such as IL-2, IL-4, IFN-γ, IL-12 and lymphotoxin, but they enhanced the production of inhibitory cytokines such as IL-10 and TGF-β. Moreover, imipramine and mianserin decreased the IFN-γ/IL-10 ratio. These *in vitro* effects were obtained at 1 μM concentrations, i.e. in the range of the therapeutic plasma concentrations achieved during clinical treatment with those antidepressants. The results suggest that TCAs and HCAs may have negative immunoregulatory effects as they inhibit stimulatory cytokines while enhancing inhibitory cytokine production. Our findings are in agreement with those of Xia et al. [48], who detected that TCAs significantly inhibited IL-2 secretion by T lymphocytes and TNF-α by activated monocytes isolated from the blood of healthy subjects. The results also corroborate the findings of Maes et al. [26], who detected that the tricyclic drug, clomipramine (10^{-6} M) inhibited IFN-γ and enhanced IL-10 production and those of Kubera et al. [16], who obtained similar results using imipramine, despite the fact that those authors used whole blood cell cultures instead of

PBMC isolated from the blood, used in our experiments. Also splenocytes of stress-exposed and imipramine-treated rats exhibited a decrease in the ability to produce IL-1 and IL-2 [17].

There are no papers concerning the influence of mianserin on Th1 or Th2-like cytokine production in PBMC, but our results indicated that despite the differences in the chemical structure, mianserin and imipramine exerted a similar effect on unstimulated and PHA + LPS-stimulated cytokine production. It is worth mentioning that the effect of both antidepressants on expression of brain-derived neurotrophic factor (BDNF) was also comparable [29].

The exact mechanism by which antidepressants affect stimulated cytokine production in leukocytes is still unknown. These effects may be mediated by non-specific inhibitory effects on lymphocyte blastogenesis, DNA synthesis, or alterations in second messengers. Chronic antidepressant administration may cause alterations in cAMP synthesis or increase in the expression of cAMP response element binding protein (CREB), as it was detected in the rat hippocampus, and lead to the regulation of specific target genes, such as BDNF mentioned above, demonstrating a role of cAMP cascade in the action of antidepressants [30, 47]. The immune effects of TCAs and HCAs may also be partially explained by their serotonergic activities, such as the depletion of intracellular serotonin stores or serotonergic receptor blockade, which in turn may suppress various aspects of the inflammatory response system, T cell proliferation and the production of Th1-like cytokines [42, 50, 51].

We expected that antidepressants may induce apoptosis of Th1 lymphocyte population and by this mechanism decrease the production of Th1-like cytokines. However, imipramine and mianserin at concentrations which inhibited cytokine production were not toxic and did not induce apoptosis of PBMC, so this possibility was excluded.

Major depression is associated with an increased secretion of IFN-γ [23, 37]. Moreover, administration of IFN-γ results in behavioral effects and depressive-like syndromes [43]. Because antidepressants decrease IFN-γ production but enhance the production of IL-10, which is an inhibitor of IFN-γ synthesis and action, one may speculate that antidepressants exert some of their antidepressant effects through their negative immunoregulatory capacities. Moreover, we detected that production of another inhibitory cytokine, TGF-β, was significantly enhanced by both antidepressants. This cytokine is known to inhibit several immune reactions, especially in autoimmune disorders. On the other hand, it can stimulate the activity of some immune cells such as phagocytes and also regulate neuron survival or death, so the role of its enhanced production under the influence of antidepressants should be examined in the future experiments [13, 14].

In our study, imipramine and mianserin significantly inhibited IL-12 production in PBMC. Since IL-12 is known as a factor positively influencing IFN-γ production in Th1 lymphocytes [32, 46], the negative influence of both antidepressants on Th1-like cytokine production can be also considered as the effect of the inhibition of IL-12 synthesis and release.

As it was mentioned above, TCAs inhibit the production of TNF-α [47], which is mainly the product of monocytes/macrophages. In our study, we examined the influence of imipramine and mianserin on lymphotoxin, which is the product of Th1 lymphocytes, and detected that its production was also significantly inhibited by both antidepressants. To our knowledge, the influence of imipramine and mianserin on lymphotoxin was not examined, but rolipram, known as an antidepressant and phosphodiesterase inhibitor, suppressed not only the production of IFN-γ or TNF-α but also lymphotoxin in autoreactive T cells from rats with autoimmune encephalitis and in humans with multiple sclerosis [44], so it seems likely that the expression of both cytokines may be regulated negatively by antidepressants.

In contrast to the mainly inhibitory effect of both antidepressants on Th1-like cytokine production, their influence on Th2-like cytokines varied as they enhanced IL-10 but suppressed IL-4 production. These data are in agreement with the results of other authors [15] who detected that splenocytes isolated from mice treated for a relatively short time (2 weeks) with desipramine (active metabolite of imipramine) produced *ex vivo* significantly less IL-4 than the cells of mice not treated, while the enhancement of IL-10 production by desipramine changed gradually with the prolonged administration of the drug.

Apart from imipramine and mianserin, also lithium used at a therapeutic concentration significantly enhanced the unstimulated production of IFN-γ and IL-10. Moreover, it also enhanced the production of IL-2. It has already been reported

that lithium may induce IL-2 and sIL-2R *in vivo* in healthy volunteers, and *in vitro* it may trigger non-induced human PBMC to release some cytokines, such as TNF-α and IL-10, and whole blood cell cultures to release IL-8, but the mechanism by which lithium can act as an inducer of cytokine production is unknown [18, 19, 44, 47, 49].

Lithium is a simple monovalent cation, which, as a salt, is used in the treatment of mania, in augmentation treatment of depression, and in the prophylaxis of manic-depressive illness. *In vivo* and *ex vivo* studies demonstrate that lithium attenuates cAMP activity and by decreasing cAMP it can enhance the production of Th1-like cytokine production [34].

In our study, lithium also elevated PHA + LPS-stimulated production of IFN-γ, IL-10, as well as IL-2 in PBMC of healthy subjects. These results confirmed the results of other authors [18, 25] that lithium enhanced IL-2 after induction with mitogens and increased the stimulated and unstimulated production of negative immunoregulatory IL-10, which is known to exert a potent inhibitory activity on monocytic and Th1-like functions including the production of cytokines probably by influencing NFkB activation pathway [19]. Moreover, treatment with IL-10, which is not an antidepressant drug, may block the depressive-like behavioral symptoms which occur in rodents treated with LPS [3, 12], so enhancement of IL-10 production can be the part of therapeutic activity of lithium.

It seems likely that lithium induces a shift in the immune system towards anti-inflammatory cytokine secretion not only by the stimulation of IL-10 production or other inhibitory cytokines such as IL-1RA [25], but also by enhancing the production of other pleiotropic anti-inflammatory cytokines such as TGF-β. A significant increase in its production by PBMC treated with polyclonal activators in the presence of lithium was detected in our study. In depressed patients treated with lithium, a significant reduction of positive acute AP proteins was detected [1] along with an increase in serum level of soluble IL-2R (sIL-2R) [36, 41]. As sIL-2R forms inactive complex with IL-2, its increase can also be considered as immunosuppressive action of lithium [5]. In our experiments, lithium also significantly inhibited production of IL-4. This result is inconsistent with that reported by Rapaport and Manji [35], who after 5 days of treatment of whole blood cell cultures with a mitogen in the presence of lithium, detected a significant increase in IL-4 production together with IL-10, but a significant decrease in Th1-like cytokines, such as IL-2 and IFN-γ. We can only speculate that these discrepancies are caused by the differences in the type of cell culture or in the time of cytokine production.

Not all cytokines examined in our study were influenced by lithium. No significant changes were detected in stimulated IL-12 and lymphotoxin production. These data strongly suggest that lithium can exert a varied influence not only on Th1 or Th2-like cytokine production but also on cytokines produced mainly by monocytes/macrophages.

In our study, we also compared the influence of a TCA imipramine, HCA mianserin and mood stabilizer lithium on ROS production in noninduced and phorbol ester (PMA)-induced neutrophils isolated from blood of healthy subjects. When imipramine and mianserin were used at doses within the therapeutic range, no influence of antidepressants on superoxide anion production in neutrophils *in vitro* was observed. However, both imipramine and mianserin, when used at a high concentration of 100 μM, inhibited noninduced and PMA-induced superoxide anion production. It has already been shown that imipramine causes a concentration-dependent decrease in the chemiluminescence of activated macrophages during phagocytosis [11]. Moreover, imipramine was shown to inhibit lipid peroxidation both in *ex vivo* and *in vitro* study of the rat cerebral cortex [28]. We can extend those observations, because in our experiment mianserin was also a strong inhibitor of superoxide accumulation in noninduced neutrophils *in vitro*, in comparison to imipramine.

In contrast to imipramine and mianserin, lithium used at low doses of 1 mM or 100 μM (the doses within the therapeutic range) did not change the superoxide anion concentration. In the reports of other authors [11], lithium enhanced nonstimulated superoxide production, however, when used at 5 mM concentrations, higher than used in our study. In the case of FMLP-stimulated superoxide production, 5 mM lithium down-regulated superoxide production. It should be stressed that we used neutrophils of healthy persons and their reactivity to lithium treatment can differ significantly from those of patients with major depression, in whom overactivation of neutrophils and enhancement of the "oxidative burst" was described [7]. It seems likely that *in vivo* lithium can modulate the activity of neutrophils of persons with major depression *via*

inositol phospholipid second messenger generating system [4].

It seems likely that lithium may have different effects on depressed patients and healthy volunteers: in cells of depressed patients, lithium may act to normalize phosphoinositol activity while in normal volunteers it can stimulate cell activity, or at lower concentrations, it may have no influence on ROS production.

Summing up, all examined antidepressants regulated production of cytokines in PBMC mainly by increasing the production of anti-inflammatory ones and inhibiting pro-inflammatory. Imipramine and mianserin used at high concentrations also inhibited the "oxidative burst" in neutrophils isolated from blood of healthy volunteers.

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