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## SHORT COMMUNICATION

# INFLUENCE OF ANTIDEPRESSANT DRUGS ON MACROPHAGE CYTOTOXIC ACTIVITY IN RATS

*Dariusz Belowski*[#], *Jan Kowalski, Andrzej Madej, Zbigniew S. Herman*

Department of Clinical Pharmacology, Silesian University School of Medicine, Medyków 18, PL 40–752 Katowice, Poland

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The aim of the study was to evaluate the *in vivo* and *in vitro* effects of antidepressant drugs on cytotoxic activity of rat spleen macrophages. In the *in vivo* experiment, rats were injected subcutaneously with two different doses (2 or 10 mg/kg) of desipramine, fluvoxamine and fluoxetine. The drugs were given once, for 2, 4 or 8 weeks. In the *in vitro* experiment, spleen macrophages were cultured with three different concentrations of desipramine (3.75, 0.75, or 0.075 mM), fluvoxamine (3.14, 0.62, or 0.062 mM), and fluoxetine (3.23, 0.64, or 0.064 mM) for 72 h. The cytotoxic activity of macrophages was evaluated by measuring the lysis of ($^{51}$Cr) chromate-labelled P-815 target cells. In the *in vivo* experiment, a single dose of fluvoxamine (2 and 10 mg/kg) and fluoxetine (10 mg/kg) significantly decreased macrophage cytotoxic activity. Fluvoxamine (2 and 10 mg/kg), fluoxetine (10 mg/kg) and desipramine (10 mg/kg) administrated for 14 days also decreased macrophage cytotoxic activity. Twenty-eight day treatment with desipramine (2 and 10 mg/kg) decreased macrophage cytotoxic activity. Desipramine, fluvoxamine and fluoxetine given for 56 days did not affect macrophage cytotoxic activity. In the *in vitro* experiment, antidepressant drugs did not affect the cytotoxic activity of macrophages. The results of the study indicate that the effects of antidepressant drugs on macrophage cytotoxic activity depend on the drug type, dose and duration of the treatment.

***Key words:*** *macrophages, cytotoxic activity, desipramine, fluvoxamine, fluoxetine*

[#] *correspondence;*

## INTRODUCTION

The internal homeostasis of the body is under the influence of exogenous and endogenous conditions, and its maintenance is possible thanks to the cooperation of the nervous, endocrine and immune systems [1, 8]. Although experimental studies indicate that psychotropic drugs modify the functions of immune cells [16, 22, 31], data concerning the effects of antidepressant drugs on the cytotoxic activity of macrophages are scarce. Macrophages belong to the mononuclear phagocyte system including blood monocytes and tissue macrophages residing in different organs. Macrophages play an essential role in the immune response: they are phagocytes, they present antigen in the induction phase and kill cells in the effectory phase of the immune response. They also regulate the functions of other immune cells [18, 26, 28]. Clinical studies performed in the last 20 years have shown alterations of the activity of the immune system in depressive patients [21, 33]. This may suggest disturbances in the functional interrelations between the central nervous system and the immune system. It is noteworthy that in depressive patients, the cell-mediated immunity is impaired, resulting in an increased incidence of infectious and neoplastic diseases in these patients [13, 27]. To date, few clinical studies have been performed to evaluate the effect of antidepressant therapy on the immune system, and their results are controversial [2, 4, 27]. The aim of this study was to evaluate the effects of some antidepressant drugs on the cytotoxic activity of macrophages. In the study, we used an animal model with a short- or long-term antidepressant treatment.

## MATERIALS and METHODS

### Animals

Male Wistar rats weighing 180 g were obtined from the Animal Farm of the Silesian University School of Medicine. They were kept 9 per cage at room temperature and under natural light conditions. They recived a standard rats chow and water *ad libitum*.

### Drugs

Desipramine was purchased from Ciba-Geigy, fluvoxamine was from Solvay-Duphar (Netherlands) and fluoxetine from Ely Lilly (USA). ($^{51}$Cr) chromate used in cytotoxicity assay was provided by DuPont NEN (USA).

### Culture medium

Culture medium RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Gibco), 2 mM glutamine, 100 μg/ml of streptomycin, 100 UI/ml penicillin, designated as the complete medium, was used.

### Animal treatment (*in vivo* experiment)

In the *in vivo* experiment, antidepressants were dissolved in 0.9% NaCl. Rats were injected with subcutaneous doses (2 or 10 mg/kg) of desipramine, fluvoxamine and fluoxetine. Antidepressant drugs were given once, for 14 or 28 days. The rats were decapitated after the last dose and their spleens were isolated to obtain macrophages.

### *In vitro* experiment

In *in vitro* experiment, spleen cells were isolated from untreated rats. Macrophages were isolated as described above, and then were cultured in the presence of antidepressants for 72 h. The drug concentrations were as follows: desipramine 3.75, 0.75, and 0.075 mM, fluvoxamine 3.14, 0.62 or 0.062 mM, and fluoxetine concentrations were 3.23, 0.64 or 0.064 mM. Cell cultures without drugs served as controls. After 72 h the cells were harvested, rinsed, and counted. The cytotoxic activity of macrophages was evaluated as described below.

### Cytotoxicity assay

Spleens of antidepressant-treated and control rats were excised under sterile conditions. A spleen cell suspension was prepared using a cell dissociation sieve-tissue grinder (Sigma). Mononuclear cells were seperated by gradient contrifugation using lymphoprep (Nyegaard, Oslo). Then, a splenocyte layer was removed, resuspended in complete medium, washed 4 times, placed in 96-well microtiter plate (Flow-Linbro) and incubated for 2 h (37°C, 5% $CO_2$, 80% humidity). The adherent cell fraction consisted of 86–89% of macrophages/monocytes, 8% of lymphocytes and 3–6% of granulocytes. Adherent and non-adherent cells were stained by the May-Grünwald-Giemsa method. Adherent cells mainly macrophages/monocytes were

gently washed and used for cytotoxic study. P-815 cells were used as taget cells in the macrophage cytotoxicity assay. They were incubated in complete medium for 2 h with 200 μCi ($^{51}$Cr) chromate, and then washed 4 times and mixed with effector cells at different ratios. Macrophage/monocyte activity was measured after 16 h of incubation. After incubation, experimental ($^{51}$Cr) chromate release (ER) was measured in 100 μl of supernatant. Maximal ($^{51}$Cr) chromate release (MR) was defined as the release after addition of 100 μl of 1% sodium dodecyl sulfate. Spontaneous release (SR) was measured in ($^{51}$Cr) chromate-labeled target cells incubated in complete medium alone.

Specific lysis was calculated as follows: The ratios of effector cells (E), i.e. adhering cells, to target cells (T) were as follows: 100:1, 50:1, and 25:1. The cytotoxic activity of macrophages was calculated according to the following formula:

$$CA\% = (ER–SR/MR–SR) \times 100,$$

where CA% is macrophage cytotoxic activity, ER is ($^{51}$Cr) chromate release from target cells lysed by macrophages, SR is spontaneous ($^{51}$Cr) chromate release from intact target cells, MR is maximum ($^{51}$Cr) chromate release from completely lysed target cells.

### Statistical analysis

Differences between control and treatment groups were ststistically analyzed by Student's *t*-test with $p < 0.01$ considered as significant. The results are means of three experiments (three measurements in each experiment).

## RESULTS

The percentage of macrophages/monocytes in a spleen cell suspension was 30%. After separation, the percentage of macrophages/monocytes in the population of adhering cells was about 90%. After 16 h of incubation, spontaneous cytotoxicity against P-815 target cells was 24–30% in all experiments. When spontaneous ($^{51}$Cr) chromate release exceeded 30%, then all results of such an experiment were discarded. In the *in vitro* experiment, the percentage of dead cells after 72-hour incubation ranged from 12 to 15%, and it was not altered by the studied drugs. None of the drug concentrations used in the *in vitro* experiment significantly affected macrophage cytotoxic activity.

In the *in vivo* experiment, a single dose of fluvoxamine (both given at 2 and 10 mg/kg) or fluoxetine (10 mg/kg) significantly decreased macrophage cytotoxic activity. A single dose of fluoxetine (2 mg/kg) or desipramine (2 and 10 mg/kg) had no significant effect on macrophage cytotoxic activity.

Fourteen day administration of fluvoxamine (2 and 10 mg/kg), fluoxetine (10 mg/kg), and desipramine (10 mg/kg) significantly decreased macrophage cytotoxic activity. Fluoxetine (2 mg/kg) and desipramine (2 mg/kg) given for 14 days had no significant effect on macrophage cytotoxic activity.

After 28 days of desipramine treatment (2 and 10 mg/kg) macrophage cytotoxic activity decreased significantly. Two different doses of fluvoxamine and fluoxetine given for 28 days produced no significant effect on macrophage cytotoxic activity.

*Table 1.* Influence of antidepressant drugs (10 mg/kg) on macrophage cytotoxic activity *in vivo*.

| Time of treatment | 1 day | | | 14 days | | | 28 days | | | 56 days | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E : T | 100:1 | 50:1 | 25:1 | 100:1 | 50:1 | 25:1 | 100:1 | 50:1 | 25:1 | 100:1 | 50:1 | 25:1 |
| Control | 45.1 ± 1.2 | 35.4 ± 0.8 | 31.6 ± 2.1 | 48.8 ± 0.8 | 38.3 ± 0.9 | 33.4 ± 2.0 | 42.6 ± 1.0 | 36.9 ± 0.9 | 31.1 ± 0.8 | 46.2 ± 0.6 | 33.5 ± 0.4 | 23.8 ± 1.2 |
| Desipramine | 45.1 ± 1.3 | 33.0 ± 2.1 | 29.8 ± 2.2 | 42.2* ± 1.2 | 34.9# ± 1.4 | 30.6# ± 0.6 | 36.8* ± 2.0 | 32.4* ± 2.2 | 26.7* ± 1.9 | 47.5 ± 2.2 | 33.4 ± 1.8 | 23.2 ± 1.1 |
| Fluvoxamine | 38.0* ± 2.2 | 27.1* ± 2.0 | 22.8* ± 1.6 | 37.2* ± 0.8 | 33.9* ± 0.1 | 28.3* ± 0.4 | 41.4 ± 1.9 | 36.3 ± 3.0 | 30.8 ± 2.1 | 48.0 ± 2.0 | 34.7 ± 1.3 | 24.7 ± 0.3 |
| Fluoxetine | 38.7* ± 0.9 | 28.1* ± 1.4 | 21.7* ± 0.6 | 39.3* ± 2.8 | 34.2# ± 1.7 | 29.7# ± 1.5 | 41.4 ± 0.9 | 36.1 ± 1.2 | 31.6 ± 1.1 | 47.6 ± 1.2 | 33.2 ± 1.4 | 24.5 ± 1.2 |

The data are expressed in percentage of cytotoxic activity ± SE as the arithmetic mean of 12 measurements performed for each drug and effector : target ratio (E : T); * $p < 0.01$, # $p < 0.05$

Desipramine, fluvoxamine, and fluoxetine (2 and 10 mg/kg) given for 56 days had no effect on macrophage cytotoxic activity in comparison with the control group (Tabs. 1 and 2).

Quantitative analysis of macrophages stained by the May-Grünwald-Giemsa method after 28 and 56 days of treatment revealed no changes in the number of macrophages compared with the control. This indicates that the modulatory effect of the antidepressants on macrophage cytotoxic activity is independent of the number of macrophages.

## DISCUSSION

Although experimental studies suggest that psychotropic substances modulate the functional parameters of the immune system [16, 22], there is no experimental and clinical data concerning the effect of antidepressants on the cytotoxic activity of macrophages. The results of our study indicate that some antidepressants affect macrophage cytotoxic activity which is one of the parameters of the cell immune response. The *in vivo* experiment has shown that changes in the activity of macrophages induced by antidepressants are related to the dose and duration of treatment. Single injection of fluvoxamine or fluoxetine decreased macrophage cytotoxic activity, while a single injection of desipramine did not. After 14 days, fluvoxamine, fluoxetine and desipramine still decreased macrophage activity. Moreover, after 28 days, desipramine still decreased macrophage cytotoxic activity. In contrast, fluvoxamine and fluoxetine administrated for 28 and 56 days and desipramine administrated for 56 days caused the return of macrophage activity to control values. However, it should be pointed out that in the *in vitro* experiment none of the used drugs had an effect on the activity of macrophages. This finding indicates that the drugs do not produce a direct effect on macrophages, and suggests that other indirect mechanisms are involved in the modulation of macrophage cytotoxic activity. To date, the mechanisms of action of antidepressants on cells involved in the immune cell response are still unclear. The modulation of the activity of spleen cells, including macrophages, is associated, among other things, with the presence of peripheral autonomic nerve endings in the spleen parenchyma [15]. This may suggest that the activity of spleen cells is modified by neurotransmitters, especially norepinephrine and serotonin. The effect of a single dose or short-term administration of antidepressants is caused by the inhibition of monoamine reuptake, which leads to an increase in the intersynaptic monoamine concentrations [11, 34]. Short-term fluvoxamine and fluoxetine treatment inhibits serotonin reuptake [25]. Acting *via* specific receptors, serotonin inhibits the activity of immune cells, including macrophages [14, 24]. It seems that the immunomodulatory effect of the studied drugs may be partially associated with adaptive changes in the activities of adrenergic and serotonergic receptors [19, 34, 37], which are also present on macrophages. This may explain an initial increase in the activity of macrophages followed by normalization during chronic treatment. Chronic desipramine or fluoxetine treatment inhibits the function of $_2$-adrenergic receptors [12]. Similarly, chronic fluoxetine treatment inhibits the function of 5-HT$_{1A}$ receptors [17]. Both adrenergic

Table 2. Influence of antidepressant drugs (2 mg/kg) on macrophage cytotoxic activity *in vivo*

| Time of treatment | 1 day | | | 14 days | | | 28 days | | | 56 days | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E : T | 100:1 | 50:1 | 25:1 | 100:1 | 50:1 | 25:1 | 100:1 | 50:1 | 25:1 | 100:1 | 50:1 | 25:1 |
| Control | 44.7 ± 0.1 | 37.0 ± 0.6 | 31.9 ± 1.6 | 35.9 ± 0.5 | 27.2 ± 0.2 | 22.7 ± 0.6 | 43.4 ± 0.9 | 34.2 ± 1.9 | 25.5 ± 1.6 | 45.9 ± 0.1 | 33.6 ± 0.5 | 26.6 ± 0.2 |
| Desipramine | 43.0 ± 0.9 | 37.3 ± 0.9 | 30.5 ± 1.4 | 34.7 ± 1.2 | 25.8 ± 1.8 | 21.4 ± 1.6 | 34.6* ± 0.4 | 26.1* ± 1.0 | 21.0# ± 1.4 | 45.9 ± 2.1 | 34.6 ± 1.8 | 25.2 ± 2.0 |
| Fluvoxamine | 40.7# ± 1.8 | 32.4* ± 0.5 | 26.8* ± 1.1 | 28.5* ± 2.0 | 21.8* ± 0.9 | 19.0* ± 1.8 | 43.7 ± 2.6 | 32.8 ± 1.8 | 25.3 ± 1.6 | 45.0 ± 2.3 | 34.7 ± 1.3 | 24.6 ± 1.1 |
| Fluoxetine | 44.5 ± 1.6 | 37.6 ± 1.1 | 31.5 ± 1.4 | 36.2 ± 0.4 | 26.1 ± 1.2 | 21.8 ± 1.2 | 44.6 ± 0.8 | 33.3 ± 2.2 | 24.8 ± 1.6 | 46.9 ± 1.2 | 34.0 ± 2.2 | 26.0 ± 1.7 |

The data are expressed in percentage of cytotoxic activity ± SE as the arithmetic mean of 12 measurements performed for each drug and effector : target ratio (E : T); * $p < 0.01$, # $p < 0.05$

and serotonergic receptors, which are also present on macrophages, belong to the group of receptors inhibiting cell activity [9, 14, 30]. Thus, the inhibited functions of these receptors due to chronic antidepressant treatment may lead to an increase in the activity of macrophages. The immunomodulatory effect of antidepressants may also result from the action of these drugs on the neurohormonal hypothalamus-pituitary axis [29, 35]. Neurohormones seem to play an important role in the regulation of immune cell activity [20, 35]. First, chronic fluoxetine treatment inhibits the release of adrenocorticotropic hormone [23], one of the strongest endogenous immunosuppressants [3]. Second, chronic fluvoxamine or fluoxetine treatment increases the release of thyrotropic hormone [6, 10] which stimulates immune cells. The fact that chronic antidepressant treatment shifts the hormonal equilibrium in favor of hormones stimulating immune cells may be another explanation of the results of our study. The increase in the activity of macrophages induced by chronic antidepressant treatment may also result from the action of the drugs on the opioid system. Chronic desipramine or imipramine treatment increases the number of opioid receptors [5, 7]. Recently, stimulating opioid receptors have been found on macrophages [32]. Thus, chronic antidepressant treatment may activate these cells. It should be underscored that in our experimental model only long-term administration of antidepressant drugs did not decrease the activity of macrophages. From the clinical point of view, this may improve the functions of the immune system in depressive patients, whose condition improves only after several weeks of antidepressant therapy.

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