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Original research article

The influence of mianserin on TNF-α, IL-6 and IL-10 serum levels in rats under chronic mild stress

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ABSTRACT

Background: Antidepressants are known to affect the immunological system through mechanisms which are not completely understood. The aim of the present study was to evaluate the effect of the atypical antidepressant mianserin on the levels of tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6) and interleukin-10 (IL-10) in the blood of rats in an experimental model of depression.
Methods: Male Wistar rats were subjected to chronic mild stress (CMS) according to Willner's method for 6 weeks. Following the development of anhedonia, the stressed and control rats (non-stressed animals) were treated with mianserin (10 mg/kg *ip*, twice daily) for three weeks. On the last day of the experiment, a lipopolysaccharide (LPS, 100 μg/kg *ip*) was injected to mianserin- or vehicle-treated rats. TNFα, IL-6 and IL-10 levels in the blood of the rats were assayed using ELISA methods.
Results: The results indicated a significantly increased TNFα level in stressed animals when compared with the non-stressed (control) group. The levels of IL-6 and IL-10 were also elevated, especially after LPS administration. Treatment with mianserin resulted in a significant lowering of TNFα and IL-6 levels both in LPS-treated and LPS-untreated animals. There was also a decrease in IL-10 concentration in LPS-treated stressed animals.
Conclusions: The results confirm an increase in proinflammatory cytokines in the blood of rats with experimentally induced depression and show the protective role of the activity of mianserin on the cytokine levels, expressed in a lowering of TNFα and IL-6 levels in stressed animals, and of IL-10 levels after LPS administration.

Introduction

In spite of the roughly 50-year history of the therapy of depression, the treatment of this disease with antidepressant drugs still does not yield desirable effects. This is connected with our insufficient knowledge about the pathogenesis of unipolar affective disorder, and also with the multiple directions of antidepressant action. The results of numerous studies over the last few years indicate changes in the immunological system during unipolar affective disorder. Preclinical and clinical data suggest that depression is associated with the activation of the immune system, which is manifested as inflammation [11,21].

There are suggestions that in the pathogenesis of depression in patients, a key role is played by an increase in pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNFα) [27,29,30,38]. Cytokines are responsible for varied cells processes and also for the maintenance of homeostasis in tissue. The production of cytokines increases in cases of acute stress or inflammation. There is a suggestion that depression is a chronic inflammation of structures in the central nervous system responsible for emotions and mood [26]. It is known that pro-inflammatory cytokines have a direct influence on the nervous system and may cause disturbances in the functioning of the brain, resulting in behavioral changes such as anhedonia, which is one of the main symptoms of depression [34]. It is postulated that if changes in the levels of cytokines are present during depression, antidepressant drugs might reverse this effect

Abbreviations: TNFα, tumor necrosis factor alpha; IL, interleukin; CMS, chronic mild stress; LPS, lipopolysaccharide; SSRIs, serotonine reuptake inhibitors.

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[2,15,22,30,36]. The results of studies using animal models of depression confirm interactions between immunological factors and depression that can be modulated by antidepressant drugs. [13,15,36]. Although there are many studies showing the influence of antidepressants on cytokines levels, their results remain ambiguous [32]. These studies mainly concern tricyclics, a classic group of antidepressants (imipramine, amitriptiline), or selective serotonin reuptake inhibitors (fluoxetine, paroxetine, sertraline) [1,3,18,28,40], but knowledge about the action of atypical drugs like mianserin on immunological factors is still unknown. Therefore, the aim of this study was to elucidate whether mianserin, an antidepressant drug with an atypical basic mechanism of action, has an influence on immunological factors.

Materials and methods

Animals

The study material comprised 80 white male Wistar rats with a baseline body mass of 230 ± 15 g. The animals were housed in standard conditions in compartments with a temperature of 20 ± 2 °C, humidity of 60–70% and a 12 h/12 h day/night cycle. Each rat was placed in a separate cage of 40/25/15 cm. The rats received standard feed (Labofeed B) and had unlimited access to drinking water (with the exception of the time periods when the behavioral experiments took place). The animals exposed to stress were kept in a separate compartment. The animals were subjected to chronic stress according to Willner's [41] model, which is generally accepted to be the most optimal and yield the most reliable and repeatable results for factors indicative of the development of depression.

Drug and substances

Mianserin (JELFA, Jelenia Góra) as mianserin hydrochloride was dissolved in an aqua pro injectione and administered twice daily intraperitoneally (*ip*) in a dose of 10 mg/kg according to Sun and coworkers [39]. Animals serving as control groups received a proper volume of aqua pro injectione.

Lipopolysaccharide from *Escherichia coli* serotype 026:B6 (Sigma Chemicals Co.) was dissolved in an aqua pro injectione and administered in a single dose of 100 μg/kg. intraperitoneally (*ip*) according to Shen et al. [37]. 1% solution of saccharose was designed for a weekly test of consumption.

Chronic mild stress method (CMS)

The scheduled research took advantage of Willner's model of depression [41]. The animals were divided into two groups. One group ($n = 40$) was subjected to chronic mild stress (CMS) for 6 weeks by means of various randomly scheduled, low-intensity social and environmental stressors (in a week-cycle for 6 days animals were exposed to only one factor per day followed by a day with no stress): no access to food and water, reversed light/dark cycle, exposure to stroboscopic lamp with frequency of 150 flashes/min, a periodical tilt of cages under 45°, pouring water (200 ml) on rats, and paired housing. The development of the main symptom of depression, anhedonia, was controlled by a weekly test of consumption of a 1% solution of saccharose. Bottles with saccharose solution were weighed before and given to rats. After an hour of sucrose intake, the bottles were weighed again. Before this test, the animals were deprived of food and water for 12 h. The same sucrose intake test was carried out for non-stressed animals ($n = 40$).

Antidepressant effect of mianserin on rats under CMS

Following the development of anhedonia, after 6 weeks of exposing animals on stressors in CMS, stressed (S) rats were divided into two groups where one group was administered mianserin (M) (10 mg/kg *ip*, 2× daily) and another was treated with vehicle (V) (aqua pro injectione) for 21 days. Two hours before decapitation, each group was again divided into next two subgroups, where one subgroup was administered a single dose of lipopolysaccharide (LPS) (100 μg/kg *ip*) and the remaining rats were injected with vehicle (aqua pro injectione). A similar procedure was performed with the non-stressed control (K) group of animals. After decapitation, the collected blood was centrifuged at 4000 rpm for 15 min and the serum was separated and stored at −80 °C for further measurements.

Measurement of cytokines levels

Biochemical measurements were done by means of the ELISA method (Enzyme Linked-Immuno-Sorbent Assay) using commercially available kits: "Quantikine Rat TNFα", "Quantikine Rat IL-6" and "Quantikine Rat IL-10", produced by R&D Systems, Inc., USA. These tests comprised recombinant cytokines from *E. coli* and antibodies against rats TNFα, IL-6 and IL-10. The results were calculated based on the absorbance of complex cytokines–antibodies. The concentrations were obtained from model curves with a 5, 21 and 10 pg/ml detection limit for TNFα, IL-6 and IL-10, respectively.

Statistical analysis

The results were expressed as a mean ± SEM. Statistical analyses were performed using Statistica 10.0 in Windows XP. The data were evaluated by an analysis of variance (ANOVA) and Duncan's test (*post-hoc* test) for independent and dependent variables. Only statistically significant data exceeding the significance threshold of $p < 0.05$ were taken into account.

The study was approved by the Local Ethic Committee for the Use of Laboratory Animals in Poznań, Poland (56/2005 and 8/2008).

Results

Evaluation of anhedonia in the CMS model

The development of the main symptom of depression, anhedonia, in animals subjected to chronic, mild stress was controlled by a weekly test of consumption of 1% saccharose solution (Table 1). This test was performed with groups of stressed

Table 1
Changes in 1% saccharose solution consumption in the CMS model.

Group	The quantity of 1% saccharose consumption (g)			
	Week 2	Week 4	Week 5	Week 6
Stressed rats (S), $n = 40$	31.3 ± 1.9	29.1 ± 2.7	21.6 ± 2.9*	23.4 ± 1.8*
Non-stressed rats (K), $n = 40$	35.4 ± 2.7	34.4 ± 2.2	29.4 ± 3.3	33.7 ± 3.5

Mean ± SEM.
* *vs.* group K, $p < 0.05$.

Table 2
Effect of mianserin chronic administration on 1% saccharose consumption.

Group		1% saccharose consumption (g)		
		7 days	14 days	21 days
Stressed rats (S), $n=40$	Mianserin (M), $n=20$	20.8 ± 2.11	$33.7 \pm 3.7^{\wedge}$	$36.4 \pm 2.3^{*}$
	Vehiculum (V), $n=20$	$23.4 \pm 4.5^{\#}$	25.7 ± 2.2	$18.0 \pm 2.6^{\#}$
Non-stressed rats (K), $n=40$	Mianserin (M), $n=20$	32.5 ± 5.5	25.1 ± 5.0	29.4 ± 2.0
	Vehiculum (V), $n=20$	34.2 ± 5.8	32.7 ± 6.1	32.7 ± 5.0

Mean ± SEM.
* *vs.* S + V, $p < 0.05$.
$^{\#}$ *vs.* K + V, $p < 0.05$.
$^{\wedge}$ *vs.* S + V, $p < 0.07$.

and non-stressed animals. Differences in saccharose intake between the stressed (S) and non-stressed control animals group (K) were statistically significant [main effect: ANOVA (1.76) = 7.08, $p < 0.01$] after 6 weeks of CMS.

Chronic administration of mianserin

The results indicate the alleviation of symptoms of anhedonia in those groups of animals which were treated with mianserin (Table 2). The difference in saccharose consumption between the group of stressed animals with vehiculum (S + V) and the stressed group with mianserin (S + M), when compared with the non-stressed animals, were statistically significant after three weeks of administration of the drug [interaction, ANOVA II (6.138) = 3.90, $p < 0.01$]. Even in the second week of treatment with mianserin, there was a meaningful alteration ($p < 0.07$) between the stressed animals (S + V) and the stressed group with the drug (S + M), which was especially significantly observed after the third week of drug administration ($p < 0.05$). Concerning the non-stressed groups with or without the drug, there were no statistical significant differences in the consumption of 1% sucrose solution (K + M *vs.* K + V, $p > 0.05$). Also very important was that the saccharose intake of stressed animals which were still underlying CMS without administration of mianserin was decreased (S + V) when compared with the non-stressed control group (K + V), which indicates continuous development of anhedonia ($p < 0.05$).

The influence of mianserin associated with LPS on serum TNF-α, IL-6 and IL-10 levels

Concerning the levels of the pro-inflammatory cytokines, among which is tumor necrosis factor TNFα, a general variability in concentrations of this cytokine was noticed [ANOVA (7.24) = 12.4, $p < 0.01$] (Fig. 1). In the stressed group of animals (S + V), there was a statistically significant ($p < 0.005$) increase in the TNF-α level compared to the non-stressed control group (K + V). Additional stressors such as LPS also induced production of this proinflammatory cytokine, especially in the group of stressed animals (S + V + LPS *vs.* K + V + LPS, $p < 0.01$). Chronic administration of mianserin caused a meaningful ($p < 0.005$) decrease in the concentration of this cytokine in the stressed group (S + M) compared to stressed animals without the drug (S + V). In terms of stressed groups of animals with a single injection of LPS, administration of mianserin also caused a reduction in the TNFα level without the strong involvement of LPS (S + M + LPS *vs.* S + V + LPS, $p < 0.001$).

Relating to levels of another pro-inflammatory cytokine, IL-6, there was also a general alteration [ANOVA (7.28) = 20.0, $p < 0.05$] in concentrations of this cytokine (Fig. 2). The level of IL-6 in the serum of stressed animals with a single injection of LPS (S + V + LPS) was definitely higher ($p < 0.001$) than in stressed animals (S + V) without LPS. The administration of the drug only (S + M) led a decrease in the level ($p < 0.05$) of IL-6 when compared with the proper control group (S + V). Concerning the action of LPS in mianserin-treated rats, LPS did not significantly affect the level of IL-6 when compared with the drug effect ($p > 0.05$). A similar effect was noticed in non-stressed groups treated with mianserin and LPS (K + M + LPS *vs.* K + V + LPS, $p < 0.001$) and in animals treated only with mianserin (K + M *vs.* K + V, $p < 0.05$).

Concerning the levels of the anti-inflammatory cytokine interleukin 10, concentrations of this cytokine revealed general variation [ANOVA (7.62) = 2.15, $p < 0.05$] (Fig. 3). The concentration of IL-10 in the group of stressed animals was insignificantly decreased (S + V) in comparison with the non-stressed control

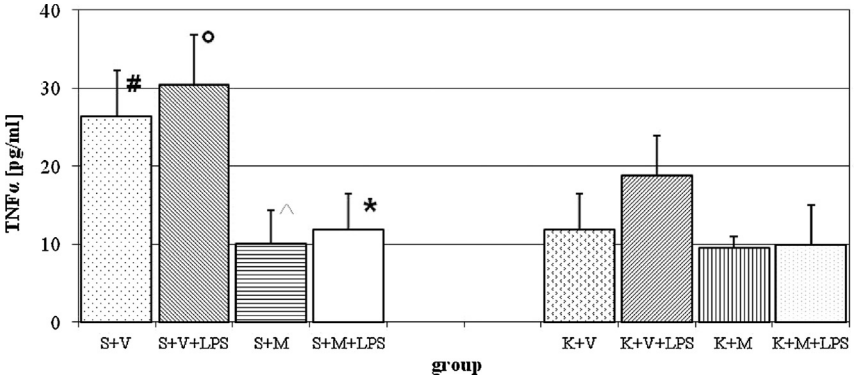

Fig. 1. The influence of chronic (21 days) mianserin (10 mg/kg *ip*, 2× daily) administration and single lipopolysaccharide (LPS) injection (100 μg/kg *ip*) on the concentration of TNFα in the blood of stressed and non-stressed rats. S + V – stressed rats receiving aqua pro injectione; S + V + LPS – stressed rats receiving aqua pro injectione + LPS; S + M – stressed rats receiving mianserin; S + M + LPS – stressed rats receiving mianserin + LPS; K + V – non-stressed rats receiving aqua pro injectione (control); K + V + LPS – non-stressed rats receiving aqua pro injectione + LPS; K + M – non-stressed rats receiving mianserin; K + M + LPS – non-stressed rats receiving mianserin + LPS; ^*vs.* S + V, $p < 0.005$; **vs.* S + V + LPS, $p < 0.001$; $^{\#}$*vs.* K + V, $p < 0.005$; ^{O}*vs.* K + V + LPS, $p < 0.001$.

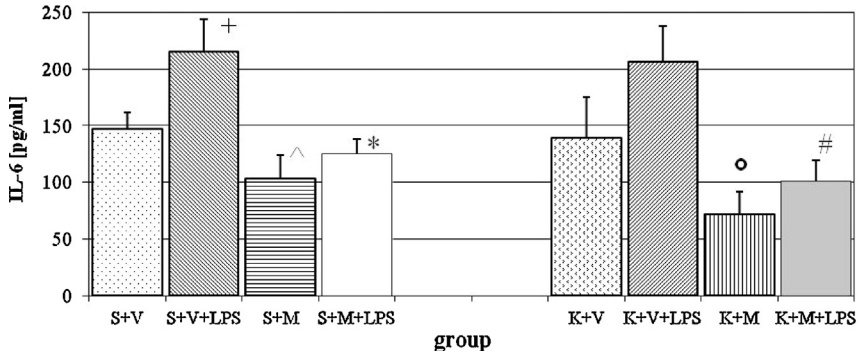

Fig. 2. The influence of chronic (21 days) mianserin (10 mg/kg *ip*, 2× daily) administration and single lipopolysaccharide (LPS) injection (100 μg/kg *ip*) on the concentration of IL-6 in the blood of stressed and non-stressed rats. S + V – stressed rats receiving aqua pro injectione; S + V + LPS – stressed rats receiving aqua pro injectione + LPS; S + M – stressed rats receiving mianserin; S + M + LPS – stressed rats receiving mianserin + LPS; K + V – non-stressed rats receiving aqua pro injectione; K + V + LPS – non-stressed rats receiving aqua pro injectione + LPS; K + M – non-stressed rats receiving mianserin; K + M + LPS – non-stressed rats receiving mianserin + LPS; $^{+}$*vs.* S + V, $p < 0.001$; ^*vs.* S + V, $p < 0.05$; **vs.* S + V + LPS, $p < 0.001$; ^{O}*vs.* K + V, $p < 0.05$; $^{\#}$*vs.* K + V + LPS, $p < 0.001$.

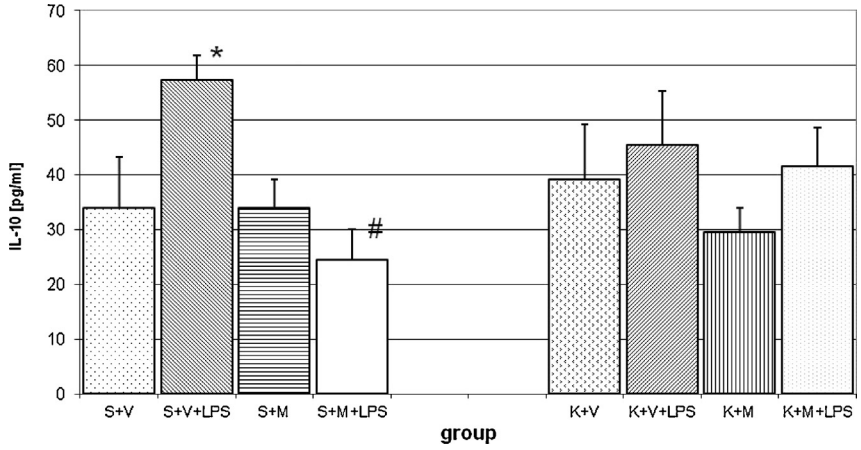

Fig. 3. The influence of chronic (21 days) mianserin (10 mg/kg *ip*, 2× daily) administration and single lipopolysaccharide (LPS) injection (100 μg/kg *ip*) on the concentration of IL-10 in the blood of stressed and non-stressed rats. S + V – stressed rats receiving aqua pro injectione; S + V + LPS – stressed rats receiving aqua pro injectione + LPS; S + M – stressed rats receiving mianserin; S + M + LPS – stressed rats receiving mianserin + LPS; K + V – non-stressed rats receiving aqua pro injectione; K + V + LPS – non-stressed rats receiving aqua pro injectione + LPS; K + M – non-stressed rats receiving mianserin; K + M + LPS – non-stressed rats receiving mianserin + LPS; **vs.* S + V, $p < 0.05$; $^{\#}$*vs.* S + V + LPS, $p < 0.001$.

group (K + V). A single administration of LPS caused an increase in IL-10 concentration in the stressed animals (S + V + LPS) in comparison with the non-stressed control group (K + V + LPS), but this difference was not statistically significant. There was a statistically significant difference ($p < 0.05$) in the levels of IL-10 between the stressed group with LPS (S + V + LPS) and proper control animals (S + V). Chronic administration of mianserin (S + M) had no effect on the IL-10 level when compared with the proper control (S + V). Moreover, in stressed animals treated with mianserin and LPS (S + M + LPS), mianserin protected the animals against the effects of LPS injection (S + M + LPS *vs.* S + V + LPS, $p < 0.001$) by lowering the concentration of IL-10 to the same level as found in the mianserin-treated animals (S + M) ($p > 0.05$). In the case of non-stressed animals, LPS caused an increase in IL-10 levels (K + V + LPS *vs.* K + V, $p > 0.05$). Mianserin treatment led to a decrease in the IL-10 level, however, the difference did not reach a statistically significant level in either the group with a single dose of LPS (K + M + LPS *vs.* K + V + LPS, $p > 0.05$) or in that without the administration of a stressor (K + M *vs.* K + V, $p > 0.05$).

Discussion

In the present study, a model of chronic mild stress (CMS) was performed, which is one of the commonly used in animal models of affective unipolar disorder [41]. A decrease in the 1% saccharose intake was observed from the fifth week of examination in stressed animals when compared with a non-stressed control group. This led to the conviction that the animals under stress displayed a state similar to anhedonia, the main symptom of depression. A 21 days administration of the antidepressant drug mianserin twice a day at a dose of 10 mg/kg caused an increase in 1% saccharose consumption after three weeks. Therefore, it can be stated that mianserin reversed the state of anhedonia in this model, which is similar to the results received by Rygula et al. [35]. These resulting changes obtained after three weeks in the state of anhedonia were reminiscent of the action of classical tricyclic antidepressants [21] or SSRIs [3,19].

The results of this research revealed significantly elevated levels of TNFα in groups of animals under chronic mild stress compared to non-stressed control groups, and also in the case of animals subjected to acute stress caused by LPS. The unquestionable effect of elevated IL-6 levels occurred in groups of animals after a single LPS injection. There was no such effect in groups of animals without the administration of lipopolysaccharide. It is possible that chronic mild stress does not affect significantly the concentration of IL-6. The results connected with increases in the levels of pro-inflammatory cytokines like TNFα and IL-6 in rats with anhedonia, which is one of the main symptoms of depression, are consistent with the reports of other authors [9,10,42]. There

are many analogies between acute stress reaction and acute inflammatory reaction revealed in immunological responses, increasing concentrations of proinflammatory cytokines, *e.g.* TNFα, IL-1, IL-6, IL-8, IL-18 or acute phase proteins [14]. These acute stress reactions in most cases increased immunological response, but chronic stress could have caused the decrease in this response [31].

The present research revealed that chronic mild stress caused no significant changes in the level of IL-10 in animals, which is similar to clinical results obtained by O'Brien, who noticed in patients with depression changes in pro-inflammatory cytokines, but not in IL-10 [33]. However, the use of LPS led to an increase in the IL-10 level, especially in the stressed group of animals, which confirms that lipopolysaccharide is a cytokine inducer [4,7,16,17].

Our results showed that the administration of mianserin caused a normalization of TNFα levels in stressed animals. These results are similar to those presented by Connor, who noticed this effect after desipramine [8]. Similarly, Brustolim et al. noticed this effect after administration of another antidepressant drug, bupropion, in mice [2]. Kaster et al. observed the shortening of TNFα-prolonged immobility time after administration of fluoxetine, imipramine and desipramine. They also noticed the inhibition of cytokine production [20]. Results of other studies showed that after stimulation by LPS and the application of imipramine, the synthesis and release of TNFα and IL-6 were significantly decreased [42]. In the present study, the level of IL-6 was increased, especially in groups of animals subjected to LPS, which is consistent with the results of other authors [5,6,12]. We found that the administration of mianserin caused a reduction in the levels of this immunological factor in stressed groups of animals with LPS combined treatment. Generally, it can be stated that chronic mianserin administration protected both stressed and non-stressed animals against the activity of an acute stressor, represented by LPS, since in the presence of mianserin LPS did not produce stimulatory activity expressed in an elevation of TNF and/or IL-6 levels. These results are in line with studies on the activity of fluoxetine [38] or amitriptiline [24] in which the authors showed that the drugs led to normalization of IL-6 concentrations in the blood of depressed patients. Similarly, the protective role of mianserin was shown in the case of IL-10 concentrations after LPS injection in stressed animals, whereas there was no such effect was observed in non-stressed animals.

The results concerning influence of mianserin on the IL-10 level are not in line with the studies of other authors. They found that after administrations of many drugs from the selective serotonine reuptake inhibitors (SSRIs), the levels of IL-10 were significantly decreased [1,13,18,25]. However, Kubera and coworkers noticed that after desipramine administration, the concentration of IL-10 was elevated [23]. Therefore, it can be speculated that the effect of chronic mianserin administration on the IL-10 level observed in this paper is due to a different, unique mechanism of action when compared with SSRIs and typical tricyclic antidepressant activity.

In conclusion, the obtained results regarding the protective role of the activity of mianserin on cytokine levels, especially in stressed animals, are interesting, but to explain such a mechanism of action, more detailed studies are necessary.

Conflict of interest

No conflict of interest.

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