GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Count and Phagocytic Activity of Leukocytes in Rats with Experimental Depressive Syndrome Caused by Systemic Administration of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine

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Leukocyte count decreased, relative content of neutrophils and monocytes increased, and their phagocytic activity was suppressed in rats with 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine-induced depressive syndrome at the stage of acute behavioral depression. The severity of behavioral depression inversely correlated with changes in the absolute neutrophil and monocyte counts.

Key Words: *depressive syndrome; model; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; rats; neutrophils; monocytes; phagocytosis*

We elaborated a model of dopamine deficiency-dependent experimental depressive syndrome (DS) caused by proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [7]. The adequacy of this model was proved in pharmacological experiments and in studies of REM sleep characteristics [4]. It is known that depression leads to immune disorders [2,3,12]. In patients with endogenous depression, the count of leukocytes, granulocytes, and monocytes increased [11, 14], while the total content of lymphocytes, absolute number of T and B lymphocytes [13], and neutrophil phagocytic activity decreased [9]. Previous studies on rats with MPTP-induced DS showed that the lymphocyte count, absolute and relative contents of T lymphocytes, and the absolute number of peripheral blood B lymphocytes decreased, while the concentration of circulating immune complexes increased [11]. We elaborated a method for estimating the severity of behavioral depression in rats [6]. To confirm the adequacy of MPTP-induced DS to clinical depression, we measured the contents of leukocytes, neutrophils, and monocytes and phagocytic activity of peripheral blood polymorphonuclear leukocytes in rats at various stages of experimental DS.

MATERIALS AND METHODS

Experiments were performed on 78 male albino Wistar rats weighing 320-380 g and kept in a vivarium under standard conditions, natural light-dark cycle, and free access to food and water. DS was induced by daily intraperitoneal injections of MPTP in a single dose of 20 mg/kg (1 ml/kg body weight, Institute of Pharmacology) for 14 (series I) or 13 days (series II). Control rats were injected with physiological saline (1 ml/kg body weight). Each experimental and control groups included 27 (series I) or 12 animals (series II). MPTP was dissolved in physiological saline immediately before the experiment. The development

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of DS was confirmed by low water intake (motivation state), preference for 10% sucrose (hedonic disturbances), immobilization time (behavioral despair) and high depression index in the forced swimming test (biorhythm disturbances), and decreased body weight [1,7]. The severity of behavioral depression was scored as described elsewhere [6]. The state of rats during the last days of MPTP administration was considered as acute behavioral depression.

For estimation of neutrophils phagocytic activity (series I), the animals were decapitated 1 day (14 experimental and 14 control rats), 1 (7 experimental and 7 control rats), and 2 weeks (6 experimental and 6 control rats) after the last injection of MPTP, and the blood was collected. For evaluation of leukocyte, neutrophil, and monocyte content (series II), the blood was taken from the caudal vein 1 day, and 2 and 4 weeks after the last MPTP injection. Leukocytes were in a Goryaev chamber. Neutrophils and monocytes were counted in blood smears by routine methods. To evaluate phagocytic activity of neutrophils, the blood (0.2 ml) stabilized with 4% sodium citrate was incubated with 0.2 ml 1-day-old culture of *Staphylococcus aureus* (strain Zhaev) at 37°C for 30 min. Blood smears were air-dried, fixed with methanol for 3 min, and stained by the method of Romanovsky—Giemsa. The phagocytic number (count of neutrophils phagocytizing bacteria) and phagocytic index (mean number of bacteria per phagocyte) were estimated by microscopy.

The results were analyzed by parametric unpaired Student's *t*, nonparametric unpaired Mann—Whitney tests, and ONE-WAY ANOVA followed by Student—Newman—Keuls multiple comparison test (Primer software). Correlation between the severity of DS and changes in phagocytic activity of neutrophils or contents of leukocytes, neutrophils, and monocytes was estimated by nonparametric Spearman rank correlation and linear correlation tests (Statgraphics software).

RESULTS

In series I, there were no statistically significant differences in the severity of behavioral depression between experimental rats decapitated 1 day, 1 and 2 weeks after the last injection of MPTP (5.4±0.7, 6.6±1.1, and 7.7±0.9 points, respectively). No differences were also found between the corresponding control groups (1.4±0.8, 1.3±0.7, and 1.7±0.6 points, respectively). The severity of behavioral depression in MPTP-treated rats was much higher than in the control ($p<0.01$, Mann—Whitney test).

Phagocytic activity of neutrophils decreased in MPTP-treated rats at the stage of marked behavioral depression. These animals had lower phagocytic number and phagocytic index compared to the control (Fig. 1, *a*, *b*). The phagocytic number also decreased 1 week after the last injection of MPTP. In addition, this parameter at the stage of marked behavioral depression was lower that 2 weeks after the last injection of MPTP ($F(2.24)=7.98$, $p<0.01$, ONE-WAY ANOVA). The phagocytic number in control rats did not vary at different periods of examination. The dynamics of changes in the phagocytic index was similar in MPTP-treated and control animals ($F(2.24)=8.74$ and $F(2.24)=8.65$, respectively, $p<0.01$): 1 day after the last injection of preparations, this parameter was higher than 1 and 2 weeks later.

In series II, the count of leukocytes decreased, while the relative contents of neutrophils and monocytes increased in MPTP-treated rats at the stage of marked behavioral depression (6.5±0.88 *vs.* 0.3±0.2 points in the control, $p<0.01$, Mann—Whitney test)

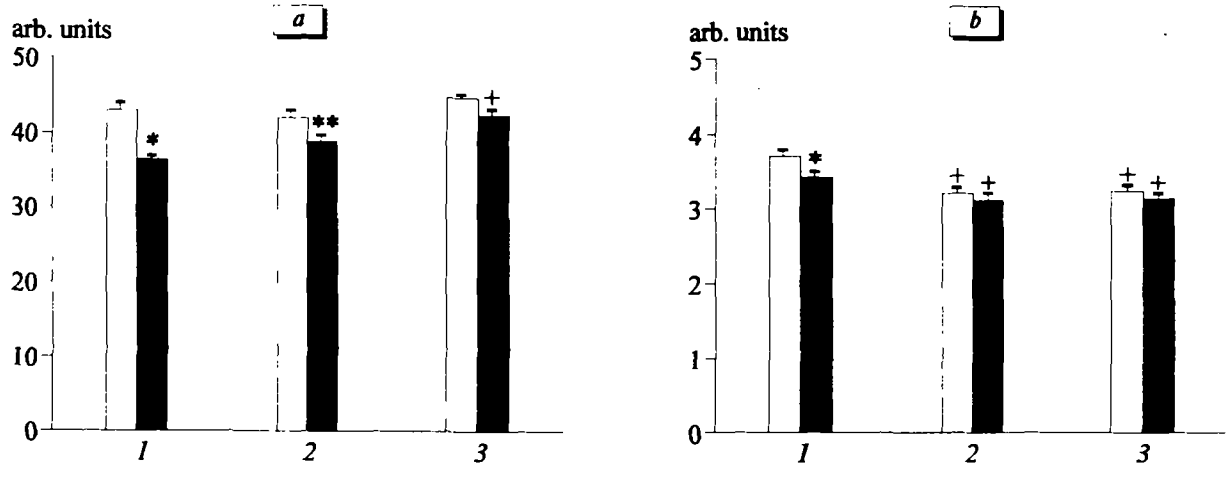

Fig. 1. Phagocytic number (*a*) and phagocytic index (*b*) in rats with depressive syndrome 1 day (*1*), 1 (*2*) and 2 (*3*) weeks after the last injection of preparations. *$p<0.001$ and **$p<0.05$ compared to the control (Student's *t* test); +$p<0.05$ compared to the parameter 1 day after the last injection of MPTP (Student—Newman—Keuls test, ONE-WAY ANOVA). Here and in Fig. 2: control (light bars) and MPTP (dark bars).

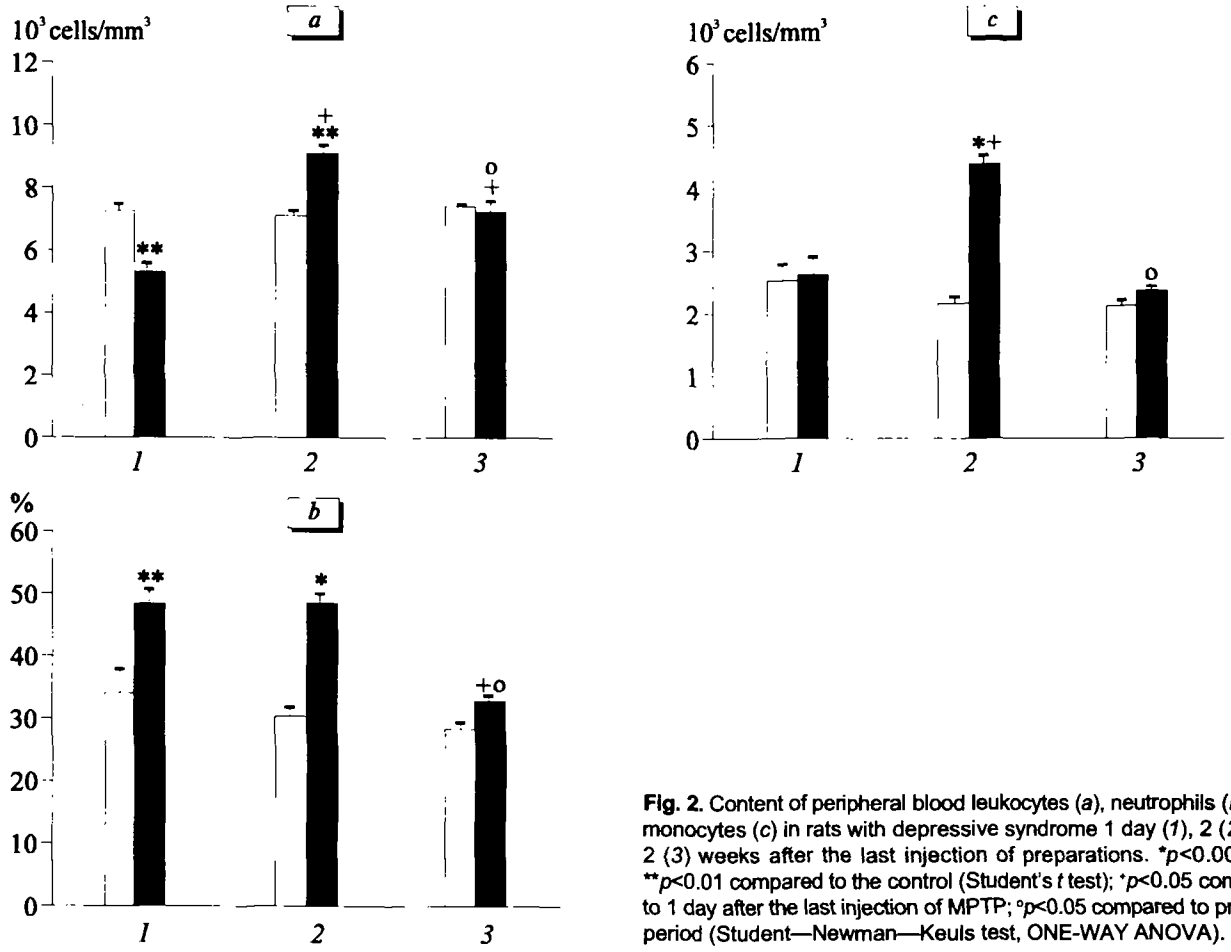

Fig. 2. Content of peripheral blood leukocytes (*a*), neutrophils (*b*), and monocytes (*c*) in rats with depressive syndrome 1 day (*1*), 2 (*2*), and 2 (*3*) weeks after the last injection of preparations. *$p<0.001$ and **$p<0.01$ compared to the control (Student's *t* test); +$p<0.05$ compared to 1 day after the last injection of MPTP; °$p<0.05$ compared to previous period (Student—Newman—Keuls test, ONE-WAY ANOVA).

(Fig. 2, *a*, *b*). Two weeks after the last injection of preparations, the count of leukocytes and absolute and relative contents of neutrophils and monocytes in experimental rats surpassed the control (Fig. 2, *a-c*). Four weeks after the last injection of preparations, there were no differences in these parameters between MPTP-treated and control animals. Changes in the content of immunocompetent cells in experimental rats attested to normalization of immunological parameters 4 weeks after the last injection of MPTP (Fig. 2). In control animals these parameters did not vary.

The severity of DS was inversely related to the phagocytic number in experimental and control rats (Table 1). Moreover, the severity of DS in control animals inversely correlated with the phagocytic index. In experimental rats, the severity of DS inversely correlated with the absolute content of neutrophils and monocytes. The leukocyte count and relative contents of neutrophils and monocytes were also characterized by high rank correlation coefficients (insignificant, $p>0.05$).

Regression analysis revealed an inverse linear correlation between the severity of DS and phagocytic number in MPTP-treated rats (linear correlation coefficient -0.56, $p<0.05$). The coefficient of linear correlation between the severity of behavioral depression and absolute contents of neutrophils and monocytes in these animals was -0.55 (insignificant). An inverse linear correlation between the severity of DS and phagocytic number or phagocytic index was also found in control rats (linear correlation coefficients -0.83 and -0.80, respectively, $p<0.01$).

Despite the fact that the phagocytic number decreased only in rats with DS, an inverse linear correlation between the severity of behavioral depression and phagocytic number was typical of both MPTP-treated and control animals. The phagocytic index changed similarly in these rats. Our findings indicate that changes in phagocytic activity of neutrophils are related to the effects of some common factors. It is known that stress leads to depression and changes immunological parameters [3,8,11]. Repeated administration of preparations probably serves as a stress factor [5]. Therefore, experimental stress can change the phagocytic activity of neutrophils. However, the interrelation between these variations and depression can not be excluded, because severe depression was shown to be accompanied by low phagocytic activity of leukocytes [9]. Phagocytic activity of blood cells

decreases in rats with experimental depression caused by removal of the olfactory bulb [10,15]. Moreover, MPTP can change phagocytic activity in rats.

The count of leukocytes, granulocytes, monocytes, and platelets increases in patients with severe depression [14]. It is believed that high content of monocytes is associated with immune dysfunction during depression. After antidepressant therapy, the content of monocytes progressively decreases. Here we studied changes in the total content of monocytes and neutrophils. In our experiments, the relative content of these cells was elevated at the stage of marked behavioral depression. Furthermore, their relative and absolute contents also increased 2 weeks after the last injection of MPTP. In rats with DS, the count of leukocytes was low at the stage of marked behavioral depression, increased 2 weeks after the last injection of MPTP, and then returned to normal. Therefore, changes in immunological parameters depend on the stage of DS.

Our findings and published data [1] indicate the development of secondary immune deficiency and low immune resistance in rats with MPTP-induced DS. These signs are typical of clinical depression. Therefore, this experimental DS corresponds to clinical depression. The search for new antidepressants and studies of their effects can be performed on the model of MPTP-induced DS. Furthermore, this model of DS holds much promise for studying changes in immunological parameters at various stages (including the early period) of depression.

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TABLE 1. Rank Correlation Coefficient for Severity of Behavioral Depression and Immunological Parameters in Rats with MPTP-Induced DS and Animals Receiving Physiological Saline 1 Day after the Last Injection

Correlation coefficient	Control	MPTP
Series I (n=14)		
Phagocytic number	-0.55**	-0.80*
Phagocytic index	-0.44	-0.71**
Series II (n=10)		
Leukocyte count/mm^3	-0.57+*	0.07
Neutrophil and monocyte contents relative	-0.58+	-0.43
absolute	-0.75**	-0.13

Note. *p<0.01, **p<0.05, +p=0.08, and +*p=0.09.

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