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EFFECTS OF BACTERIAL LIPOPOLYSACCHARIDE EXPOSURE ON IMMUNE RESPONSIVENESS IN A RODENT MODEL OF PARKINSON'S DISEASE

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Abstract - The effects of lipopolysaccharide (LPS) on immune modulation in rats subjected to a right-unilateral lesion of the substantia nigra neurons by means of 6-hydroxydopamine (6-OHDA), were investigated. LPS administration (250 μg) significantly decreases the total number of leukocytes and erythrocytes, as well as the hemoglobin level in the 6-OHDA-lesioned rats. In addition, LPS administration was also associated with an increase, relative to control, in the erythrocyte indexes and the phagocytosis by neutrophils, and in blastic transformation of T lymphocytes.

The obtained data indicated that LPS exposure might represent a risk factor for the development of the immunological changes associated with Parkinson's disease.

Key words: 6-hydroxydopamine; lipopolysaccharide; leukocyte; erythrocyte indexes; phagocytosis; proliferative response of T lymphocytes.

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN) and the presence of Lewy body inclusions in residual neurons. In recent years, increasing evidence has strongly suggested a role for inflammation in the brain in the pathogenesis of PD (Sun et al., 2003). Bacterial endotoxin lipopolysaccharide (LPS) is one of the common toxins produced by Gram (-) bacteria, including *Escherichia coli*. This agent can elicit a multitude of pathophysiological effects, including inflammation, macrophage activation, fever, and septic shock (Burrell, 1994, Galanos et al., 1985). The blood-brain barrier can become leaky as a result of sepsis (Brandtzaeg et al., 1989), allowing LPS to enter the cerebrospinal fluid.

Previous studies have demonstrated that bacterial LPS exposure, which mimics Gram (-) bacterial infections, could cause a significant loss of dopamine (DA) neurons in the SN of rat. Along with DA neuron loss are the alpha-synuclein positive Lewy body-like inclusion formation and innate immunity dysfunction manifested by an increase in the number of reactive microglia, increase in proinflammatory cytokine levels, and blood-barrier leakage (Ling et al., 2006, Wang et al., 2009). Another study (Calne and Langston, 1983) suggests that acute exposure to LPS killed the DA neurons, but the percentage of loss was insufficient to compromise the DA function enough to produce symptom expression. However, this cell loss, in conjunction with the normal age-related losses of DA neurons, would eventually lead to the expression of PD symptoms in the years or decades that followed the

acute exposure. Indeed, numerous DA neurotoxins including 6-hydroxydopamine (6-OHDA) (Hritcu et al., 2008), rotenone (Sherer et al., 2003), dieldrin (Fleming et al., 1994) and paraquat (Koller and Royse, 1986) kill DA neurons in a variety of animal models. However, LPS exposure also displayed additive or synergetic effects with 6-OHDA (additive) or rotenone (synergistic) on DA toxicity (Ling et al., 2004a, Ling et al., 2004b). Animals exposed to LPS exhibit numerous indices of neuroinflammation in the nigrostriatal pathway, including life-long elevations in tumor necrosis factor alpha (TNF-α), increased numbers of activated microglia, increased levels of oxidized proteins, and reduced amounts of glutathione (GSH) (Herrera et al., 2000, Liu et al., 2000, Zhu et al., 2007).

In macrophages and neutrophils, bacteria are internalized through the process of phagocytosis (Lee et al., 2003). Phagocytic cells within the peritoneal cavity such as peritoneal macrophages, play an important role in the clearance of bacteria and bacterial components from the host peritoneum (Neal et al., 2006, Víctor et al., 1998, Yoshikawa et al., 1994).

Recent studies have shown that LPS stimulation of murine peritoneal macrophages may be important in mediating the functions that are important for bacterial killing (Víctor et al., 1998), whereas other studies have shown a decrease in phagocytic activity when macrophages are treated with LPS (Smith et al., 1998).

Therefore, the current study is designed to assess whether disordered hematopoiesis regulation via a SN neuron lesion is associated with LPS exposure, a risk factor for DA neuron loss.

MATERIALS AND METHODS

Subjects

30 male Wistar rats weighing 180 ± 50 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water *ad libitum*. The rats were divided into control (15 sham-operated animals) and LPS+6-OHDA-lesioned rats (15 animals). The rats were treated in accordance with the guidelines of Animal Bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act of Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Neurosurgery and drug administration

The rats were anesthetized with sodium pentobarbital (45 mg/kg b.w. i.p., Sigma). The SN was right-unilaterally lesioned by stereotaxic microinjections of 8 micrograms (free base) 6-OHDA (Sigma), dissolved in 4 µl physiological saline containing 0.1% ascorbic acid (Sigma), administered by Hamilton microsyringe over 4.50 min. The syringe was left in place for 5 min after injection before being slowly removed. The rats were pretreated 30 mins before the 6-OHDA infusion with 25 mg/kg i.p. desipramine (Sigma) to protect noradrenergic projections. Sham-operated rats received an injection of desipramine, followed by vehicle only in the SN. The following coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos and Watson, 2005). The lipopolysaccharide (LPS from *Escherichia coli* serotype 0111:B4, Sigma) was dissolved in pyrogen-free 0.9% NaCl to concentrations of 250 µg/kg and i.p. injected in the 6-OHDA-lesioned rats over a period of 7 consecutive days.

The hematological parameters, phagocytosis and blastic transformation of T lymphocytes were assayed after 7 consecutive days of LPS administration.

Blood sampling protocol

Blood samples were withdrawn via a Biotrol sampling catheter from the 15 sham-operated and 15 LPS+6-OHDA-lesioned rats. Blood samples (0.5 ml

approximately/sample) were collected in vials (Venosafe) containing EDTA for hematological investigations. Hematological parameters were assayed by Hematology Analyzer MS9-5 VET Automatic Full Digital Cell Counter, Melet Schloesing Laboratoires - precision instruments for hematology research.

Phagocytosis activity of neutrophils

Seven days after continuous LPS administration the phagocytic activity of the neutrophils was measured from the same blood sample used for the hematological parameters assay. The phagocytosis activity was performed *in vitro* following a previously described method (Hefco et al., 2004). Briefly, a 100 µl EDTA blood sample was mixed with 100 µl of a suspension of *Staphylococcus epidermidis* ($4x10^7$/ml) and incubated at 37°C for 30 min with slow stirring (2 rpm). Smears from the mixture of blood and bacterial culture were May Grünwald-Giemsa stained. The phagocytosis activity was calculated as the number of bacterial *Staphylococcus* phagocytized by 100 neutrophils.

Proliferative response of lymphocytes to PHA-M

The proliferative response of lymphocytes to PHA-M was tested as previously described (Hefco et al., 2004). The assay was conducted using the same blood sample collected for the phagocytic activity determinantion. In centrifuge tubes (Corning TM, FOB Sigma), 75 µl of the EDTA blood sample was mixed with 75 µl PHA-M (Sigma) and 5 ml of HAM's medium (Sigma), supplemented with 5% heat-inactivated fetal calf serum and 1% antibiotic antimicotic solution (Gibco). The final concentration of PHA-M was 8µg/ml. The tubes were incubated at 37°C in a humidified incubator with 5% CO_2. After 72 h of incubation, the samples were centrifuged at 1200 rpm for 10 min and the supernatant was removed. Smears were prepared from the sediment and were May Grünwald-Giemsa stained. Blastoid and non-blastoid lymphocytes were counted by an experimenter blind to the treatment group moving along the length of the smear from one side to the other, counting throughout the entire width, until 100 lymphocytes (blastoid and non-blastoid combined). Two slides from each sample were counted; their counts were averaged and expressed as the percent of lymphocytes that were blastoid lymphocytes, the lymphoblast proliferation index.

Histological control

The rats were killed with an overdose of sodium pentobarbital (100 mg/kg i.p., Sigma) followed by a transcardial infusion of 0.9% saline and a 10% formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution. Serial frozen sections of 50 µm thickness were cut and stained with Cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the SN were used for statistical analysis.

Statistical method

Results were analyzed with analysis of variance (ANOVA) with the lesion as the between-groups factor (SN and Control). *F* values for which $p<0.05$ were regarded as statistically significant. Number of observations was 30.

RESULTS

Histological verification

After the 6-OHDA lesions, the rats recovered quickly and gained weight by the first week. In the majority of SN-lesioned rats (8/10) the point of the syringe needle was positioned in the central part of the SN and the lesions extended to a part of the adjacent structures, including the substantia nigra pars reticulata, without any significant damage.

Effects of LPS administration on hematological parameters and the erythrocyte indexes

In the 6-OHDA-lesioned rats, LPS induced a significant decrease in the total number of leukocytes ($F(1,28)=17.27$, $p<0.009$) (Fig. 1A), erythrocytes

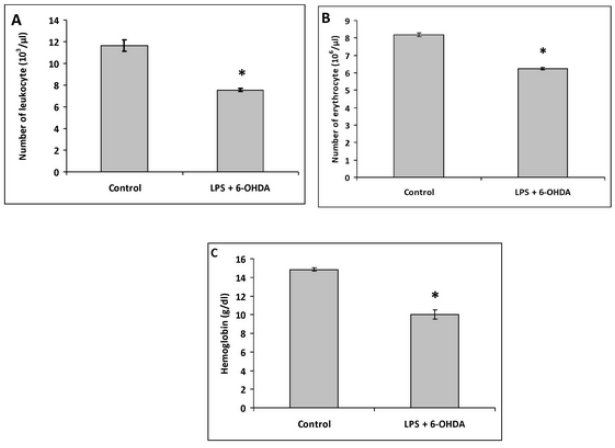

Fig. 1. a) Changes in the total number of leukocytes (*p<0.009 vs. sham-operated control group); b) The total number of erythrocytes (*p<0.006 vs. sham-operated control group); c) Hemoglobin level (*p<0.01 vs. sham-operated control group) tested after 7 continuous days of LPS administration in 6-OHDA-lesioned rats. Values are means ± SEM (n=15 per group).

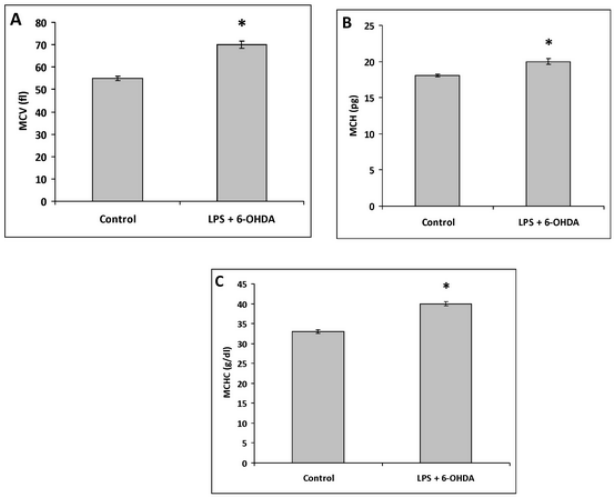

Fig. 2. a) Variation in MCV (mean cell volume) (*p<0.03 vs. sham-operated control group); b) in MCH (mean cell hemoglobin) (*p<0.02 vs. sham-operated control group); c) in MCHC (mean cell hemoglobin concentration) (*p<0.04 vs. sham-operated control group) tested after 7 continuous days of LPS administration in 6-OHDA-lesioned rats. Values are means ± SEM (n=15 per group).

(F(1,28)=40.10, p<0.006) (Fig. 1B) and hemoglobin levels (F(1,28)=19.01, p<0.01) (Fig. 1C), compared to the sham-operated groups. Additionally, LPS significantly increased the mean cell volume (F(1,28)=15, p<0.03) (Fig. 2A), mean cell hemoglobin (F(1,28)=22, p<0.02) (Fig. 2B) and mean cell hemoglobin concentration (F(1,28)=43, p<0.04) (Fig. 2C) in the 6-OHDA-lesioned rats compared with the sham-operated groups.

Effects of LPS administration on the phagocytic activity of neutrophils

In the 6-OHDA-lesioned rats, LPS induced a significant increase in the phagocytic activity of the neutrophils (F(1,18)=62.87, p<0.0001) (Fig. 3), compared with the sham-operated group.

Effects of LPS administration on blastic response of T lymphocytes

In the 6-OHDA-lesioned rats, LPS induced a significant increase the lymphoblast proliferation index (F(1,18)=80.63, p<0.0001) (Fig. 4), compared with the sham-operated group.

DISCUSSION

In a previous study we reported that the central dopaminergic system has a crucial role in the regulation of the immune processes as well as in hematopoiesis (Hritcu, 2008). Our present study demonstrated that in 6-OHDA-lesioned rats, exposure to LPS induced hematological changes in terms of the total white blood cells which support a rapid effect on the immune response in rats. A significant decrease in total white blood cells was observed 7 days after continuous LPS injection in the 6-OHDA-lesioned rats. This might be attributable to the massive migration of these cells to the sites of injection of this immunomodulator (Kitajima et al., 1995). Moreover, the LPS exposure of the 6-OHDA-lesioned rats significantly decreased the red blood cell number and hemoglobin level. We demonstrated that the rats treated with 6-OHDA and exposed to chronic LPS showed anemia. Another study suggested that nigrostriatal DA depletion rendered the animals more susceptible to bacterial endotoxin (Engler et

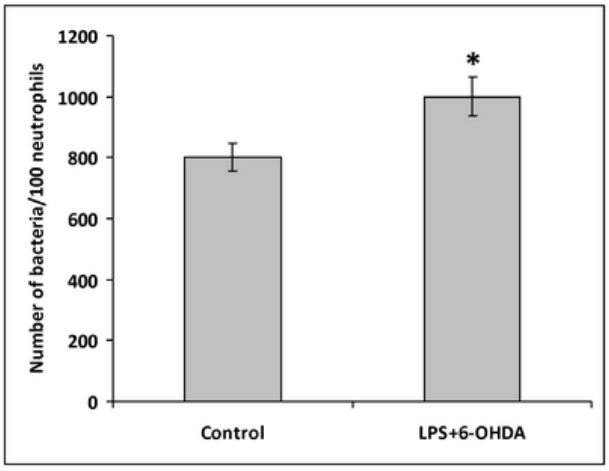

Fig. 3. Phagocytosis activity of neutrophils tested after 7 continuous days of LPS administration in 6-OHDA-lesioned rats. Values are means ± SEM (n=15 per group), *p<0.002 vs. sham-operated control group.

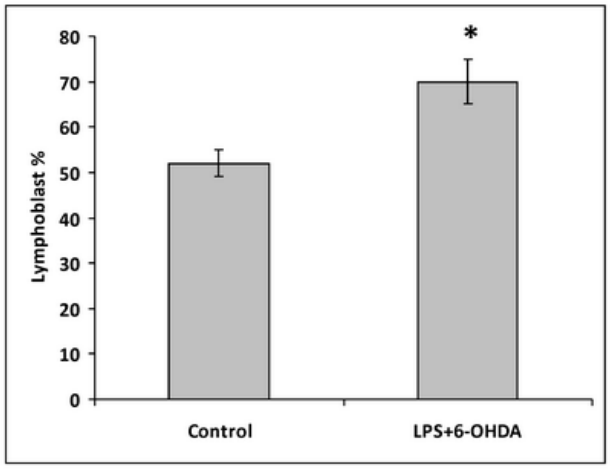

Fig. 4. The proliferative response of T peripheral lymphocytes to PHA-M tested after 7 continuous days of LPS administration in 6-OHDA-lesioned rats. Values are means ± SEM (n=15 per group), *p<0.003 vs. sham-operated control group.

al., 2009), as is demonstrated by our observation in the 6-OHDA-lesioned rats. Some studies found marked differences in the distribution and function of the blood immune cells of PD patients compared to healthy controls, whereas others reported only small differences, no differences or even changes in the opposite direction (Bas et al., 2001, Reale et al., 2009). Nevertheless, PD is often complicated with infectious diseases and the main causes of death in PD patients are pneumonia and respiratory infections (Hasegawa et al., 2000, Poewe, 2006). In our study we demonstrated that a substantial loss of dopaminergic neurons in the nigrostriatal system was associated with a significant alteration of hematopoiesis to bacterial LPS exposure. These suggest that central dopaminergic hypoactivity contributes to an increased susceptibility to infection and mortality in PD patients. Moreover, 6-OHDA-lesioned rats displayed an increase in the phagocytic activity of neutrophils and the lymphoblast proliferation index as a result of LPS exposure.

With regard to phagocytosis, this could be due to the activation of neutrophils by LPS injection (Víctor et al., 1998). Other data suggested that the chemical axotomy with 6-OHDA intensifies the proliferative response of T lymphocytes from spleen (Hefco et al., 2004). One mechanism that could explain the increased proliferative response involves catecholamine, which could act through the beta-adrenergic receptors located on the T lymphocytes surface (Hefco et al., 2004). Therefore we suggest that the increased proliferative response of the T lymphocytes in 6-OHDA-lesioned rats exposed to LPS (additive effects) is a consequence of a reduction in the sympathetic tone.

In summary, our data suggest that the hematopoiesis and abnormalities after electrolytic lesion of the central dopaminergic neurons from the SN by means of 6-OHDA are increased following LPS exposure. Additionally, macrophages play a key role during LPS exposure in 6-OHDA-lesioned rats with their most characteristic functions, showing an increase of the phagocytic process and proliferation.

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