Research Article

Hematological disorders in 6-hydroxydopamine-induced rat model of Parkinson's disease

6-hidroksidopaminle indüklenmiş Parkinson hastalığı sıçan modelinde hematolojik bozukluklar

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Abstract

Objective: The present work was undertaken in order to investigate the effects of right-unilateral lesion of substantia nigra neurons by means of 6- hydroxydopamine (6-OHDA), a dopaminergic-selective neurotoxin, on hematological parameters in rats. The primary reason for the using of rat model of Parkinson's disease was the interest regarding the role of the central dopaminergic system in hematopoiesis regulation because some neurological diseases like Parkinson's disease are well-correlated with anemia associated with autonomic dysfunction in rats.
Material and Methods: Thirty male Wistar rats weighing 200 ± 50 g at the start of the experiment were used. The substantia nigra was right-unilateral lesioned by stereotaxic microinjections of 8 micrograms (free base) 6-OHDA, dissolved in 4 µl physiological saline containing 0.1% ascorbic acid, administered through the Hamilton microsyringe over 4.50 minutes. 7 days after neurosurgery, we assessed the total number of white blood cells (WBC), the total number of red blood cells (RBC), hemoglobin level and the erythrocyte indexes (mean cell volume, MCV and mean cell hemoglobin, MCH).
Hematological parameters were assayed by a COULTER® Ac·T 5diff CP-precision instruments for hematology research.
Results: 6-OHDA treatment induced a significantly decrease of white blood cells ($p<0.03$), red blood cells ($p<0.01$), hemoglobin level ($p<0.02$) comparative with sham-operated rats. By contrast, in the 6-OHDA-lesioned rats the erythrocyte indexes (mean cell volume, MCV ($p<0.04$); mean cell hemoglobin, MCH ($p<0.01$)) were significantly enhanced comparative with sham-operated rats.
Conclusion: On the whole, the obtained data indicate the important role of the central dopaminergic system in the regulation of erythrocyte dynamics. *(Turk J Hematol 2008; 25: 140-4)*
Key words: Substantia nigra, 6-OHDA, hematological parameters.

Received: October 21, 2007 Accepted: March 18, 2008

Özet

Amaç: Bu çalışma, dopaminerjik seçimli bir nörotoksin olan 6-hidroksidopamin (6-OHDA) aracılığıyla siyah madde nöronlarının sağ tek taraflı lezyonunun sıçanlardaki hematolojik parametreler üzerindeki etkilerini araştırmak amacıyla yapılmıştır. Parkinson hastalığında sıçan modelinin kullanılmasının başlıca sebebi, hematopoez regülasyonunda merkezi dopaminerjik sistemin rolüne bağlı gelişen ilgidir; çünkü Parkinson hastalığı gibi bazı nörolojik hastalıklar, sıçanlarda görülen otonomik işlev bozukluğuna bağlı anemiyle yakından ilişkilidir.

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Gereç ve Yöntemler: Deneyin başlangıcında 200 ± 50 g ağırlığında 30 adet Wistar sıçanı kullanılmıştır. Siyah madde, 0,1% askorbik asit içeren 4 µl serum fizyolojik içinde çözülen ve 4,50 dakikadan fazla süreyle Hamilton mikroenjektörüyle uygulanan 8 mikrogramlık (serbest baz) 6-OHDA stereotaksik mikroenjeksiyon ile sağ tek taraflı olarak lezyonlanmıştır. Nöroşirurjiden 7 gün sonra, beyaz kan hücrelerinin toplam sayısını (WBC), kırmızı kan hücrelerinin toplam sayısını (RBC), hemoglobin seviyesini ve eritrosit oranlarını (ortalama hücre hacmi, MCV ve ortalama hücre hemoglobini MCH) inceledik. Hematolojik parametreler, hematoloji araştırması için COULTER® Ac·T 5diff™ CP presizyon enstrümanlarıyla analiz edilmiştir.
Bulgular: 6-OHDA uygulaması, yalancı operasyona tabi tutulmuş sıçanlarla karşılaştırıldığında, beyaz kan hücrelerinde ($p<0,03$), kırmızı kan hücrelerinde ($p<0,01$) ve hemoglobin seviyesinde ($p<0,02$) önemli ölçüde azalmayı tetiklemiştir. Bunun aksine, 6-OHDA ile lezyonlanan sıçanlarda eritrosit oranları (ortalama hücre hacmi, MCV ($p<0,04$); ortalama hemoglobin, MCH ($p<0,01$)) sahte operasyona tabi tutulmuş sıçanlara oranla büyük ölçüde artış göstermiştir.
Sonuç: Bütün olarak bakıldığında, elde edilen veriler merkezi dopaminerjik sistemin eritrosit dinamiklerinin regülasyonunda önemli bir rol oynadığını göstermektedir. *(Turk J Hematol 2008; 25: 7140-4)*
Anahtar kelimeler: Substantia nigra, 6-OHDA, Hematolojik parametreler.

Geliş tarihi: 21 Ekim 2007 Kabul tarihi: 18 Mart 2008

Introduction

Parkinson's disease is a human neurodegenerative disorder primarily characterized by a massive and progressive degeneration of the dopaminergic neurons in the substantia nigra (SN). The most widely used animal models of Parkinson's disease involve intracranial infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) directly into the ascending dopaminergic forebrain bundle, thereby inducing severe dopaminergic neuronal degeneration associated with profound deficits in feeding, drinking, and sensorimotor and learning functions [1-4]. Alternatively, new Parkinsonian rat models have been developed with 6-OHDA injected directly into the striatum to induce selective and moderate neurodegeneration of dopamine (DA) nerve terminals [5]. Similarly, in Parkinson's disease, the progressive degeneration of nigral dopaminergic neurons results in motor deficits only after 80% of the nigrostriatal system has degenerated [6]. It has been known that the brain can communicate with the immune system through either the hypothalamic pituitary (HP) axis or the sympathetic nervous system (SNS) [7,8]. The possible roles of these two major pathways in regulation of the hematopoiesis processes were examined by using pharmacological agents such as desipramine and 6-OHDA in order to determine their effects on the hematological parameters. It is also known that 6-OHDA is a useful neurotoxic agent that can reversibly impair the sympathetic nerve terminal [9,10]. When it is injected intravenously or intraperitoneally, it accumulates in the peripheral sympathetic nerve terminal and selectively destroys the sympathetic nerves. Its toxic effects directly result from its ability to generate free radical species, and from covalent bonding of quinone oxidant product [11]. With this chemical agent, we can create pure sympathectomized rats, which is reversible with the administration of desipramine, a competitive inhibitor of 6-OHDA [12]. Moreover, Bazan [13] reported the possible relationship between the nervous system and hematopoiesis. These results suggest that there is some erythropoietic regulation via the autonomic nervous system. However, the mechanism causing anemia associated with autonomic dysfunction is not well explained. Catecholamines and their corresponding receptors are widely distributed in both the central and peripheral nervous system. Besides their vasoactive effect [14], catecholamines have been known to be involved in different forms of learning and memory [4,15]. Norepinephrine (NE) particularly at the locus coeruleus (LC) area not only can regulate the hormone release from the HP axis but also the activity of the SNS [16]. Immune cell types associated with innate immunity such as natural killer cells, neutrophils, and macrophages are the potential subjects to be regulated by catecholamines because these cells express functional, β2- and/or α- adrenergic receptors [17].

In summary, the primary goal of this study was to evaluate whether disordered hematopoiesis regulation via substantia nigra neuron lesion may induce hematological disorders.

Materials and Methods

Animals

Thirty male Wistar rats weighing 200 ± 50 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22°C, 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of Animal Bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania, and all procedures were in compliance with the European 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). Right-unilateral lesioning of the substantia nigra was performed by stereotaxic microinjections of 8 micrograms (free base) 6-OHDA, dissolved in 4 µl physiological saline containing 0.1% ascorbic acid, administered through the Hamilton microsyringe over 4.5 minutes. The syringe was left in place for 5 minutes after injection before being slowly removed. The rats were pretreated 30 minutes before the 6-OHDA infusion with 25 mg/kg intraperitoneal desipramine (Sigma) to protect noradrenergic projections. Sham-operated rats received an injection of desipramine, followed by vehicle only in the substantia nigra. The following coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; and 7.4 mm ventral to the surface of the cortex [18]. Hematological parameters were assayed one week after the neurosurgery.

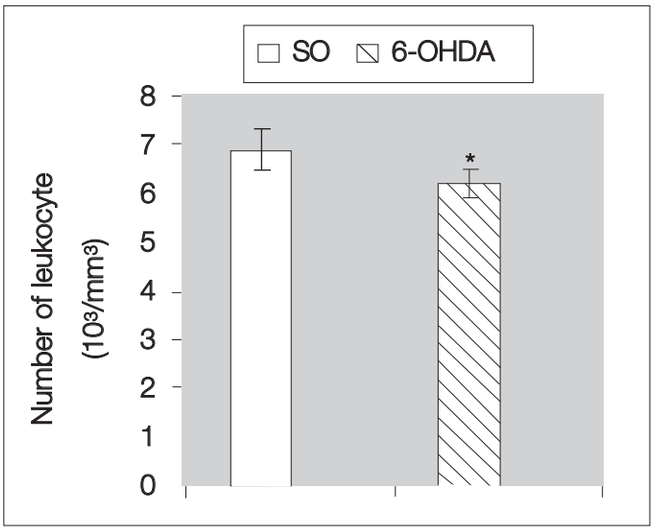

Figure 1. Changes in the total number of leukocytes tested one week after chemical lesion with 6-OHDA. Values are means ± SEM (n=15 per group). *p<0.03 vs. sham-operated (SO) group

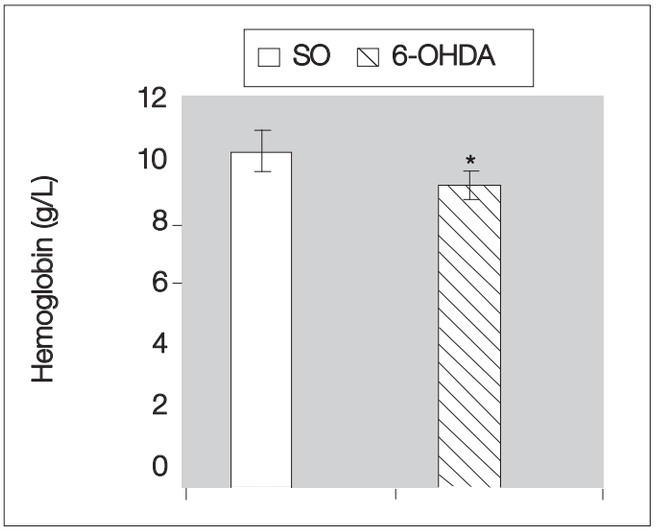

Figure 3. The effect of the chemical lesion with 6-OHDA on hemoglobin level tested one week after the neurosurgery. Values are means ± SEM (n=15 per group). *p<0.02 vs. sham-operated (SO) group

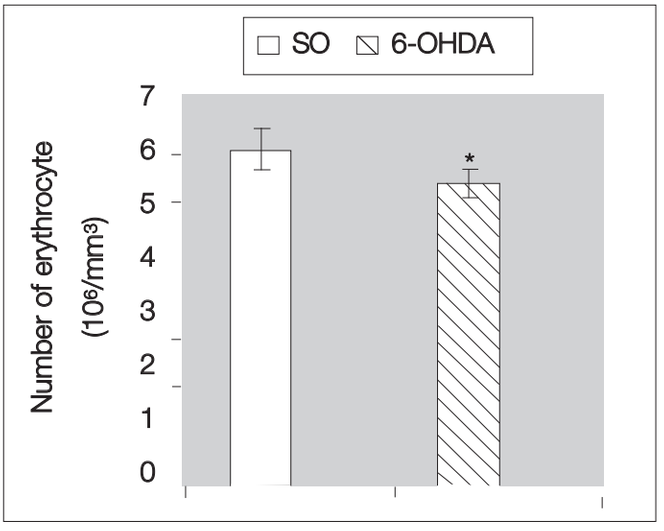

Figure 2. Changes in the total number of erythrocytes tested one week after chemical lesion with 6-OHDA. Values are means ± SEM (n=15 per group). *p<0.01 vs. sham-operated (SO) group

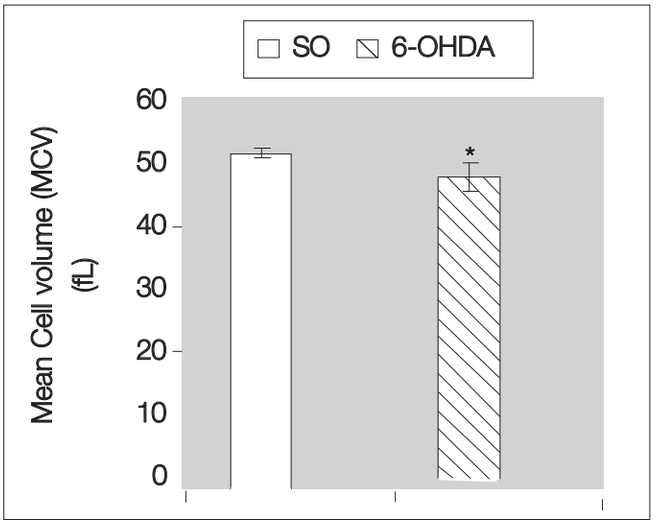

Figure 4. Variation in MCV (mean cell volume) after chemical lesion with 6-OHDA tested one week after neurosurgery. Values are expressed as mean ± SEM (n=15 per group). *p<0.04 vs. sham-operated (SO) group

Blood Sampling Protocol

One week after neurosurgery, blood samples were withdrawn via the Biotrol sampling catheter from 15 sham-operated and 15 6-OHDA-treated rats. Blood samples (0.5 ml approximately/sample) were collected in vials containing EDTA for hematological investigations.

Hematological parameters were assayed by a COULTER® Ac·T 5diff™ CP-precision instrument for hematology research.

Histological Control

At the end of the experiment, all rats were sacrificed with an overdose of sodium pentobarbital (100 mg/kg i.p.) 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. The brains were frozen and cut into coronal sections (50 μm) using a freezing microtome and stained with cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the substantia nigra were used for statistical analysis.

Data Presentations and Statistical Analysis

Results were expressed as mean ± S.E.M. Because the data were not normally distributed, the non-parametric statistic Mann-Whitney U test was employed. Results were considered significant if $p<0.05$. The number of observation was 30.

Results

Experimental data were registered one week after the 6-OHDA administration. In the 6-OHDA-lesioned rats, we observed a significant decrease in the total number of white blood cells [6.16 ± 0.3 10^3/mm^3 vs 6.8 ± 0.4 10^3/mm^3, U=36.5

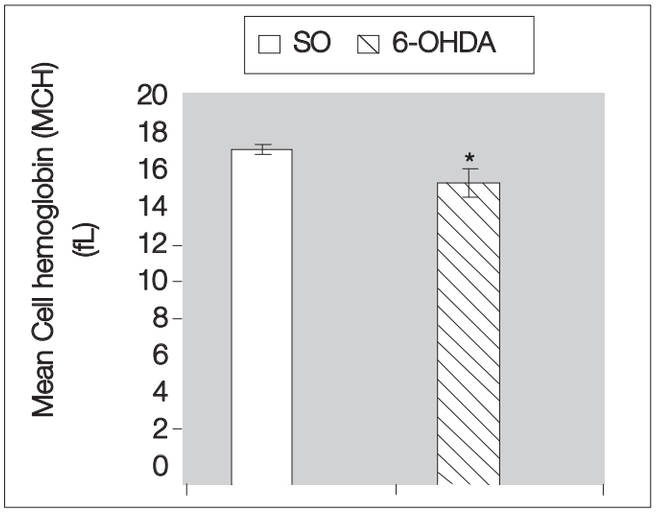

Figure 5. Variation in MCH (mean cell hemoglobin) after chemical lesion with 6-OHDA tested one week after neurosurgery. Values are expressed as mean ± SEM (n=15 per group). *p<0.01 vs. sham-operated (SO) group

p<0.03 - neutrophils (0.9 ± 0.2 vs 0.8 ± 0.2 $10^3/mm^3$), lymphocytes (4.5 ± 0.8 vs 5.6 ± 1.7 $10^3/mm^3$)] (Figure 1), red blood cells (5.01 ± 0.1 $10^6/mm^3$ vs 5.8 ± 0.2 $10^6/mm^3$, U=50.5 p<0.01) (Figure 2), and hemoglobin level (8.9 ± 0.2 g/L U=50.5 vs 9.9 ± 0.3 g/L, p<0.02) (Figure 3) compared with the sham-operated groups. 6-OHDA significantly increased mean cell volume (MCV) (51.8 ± 0.8 fL vs 50 ± 0.5 fL, U=28.5 p<0.04) and mean cell hemoglobin (MCH) (17.8 ± 0.1 pg vs 17.08 ± 0.2 fL, U=26.5 p<0.01) compared with sham-operated groups (Figures 4, 5).

Discussion

It is well recognized that the immune response is under the influence of a variety of neural or neuroendocrine mechanisms. Much less studied is the possible influence of these mechanisms on hematopoiesis.

In our previous studies, we reported that the central dopaminergic system has a crucial role in regulation of the immune processes as well as hematopoiesis [19,20]. In our present study, we used a procedure of chemical sympathectomy by lesioning the substantia nigra with 6-OHDA. By means of this particularly electrolytic lesion, we observed a significant decrease in hematological parameters registered one week after 6-OHDA administration, tested by the total number of leukocytes, erythrocytes, hemoglobin level and the erythrocyte indexes (MCV and MCH). We demonstrated that rats treated with 6-OHDA showed anemia. In addition, since the WBC significantly decreased during 6-OHDA-induced anemia, the effect of 6-OHDA in this experiment may be specific for erythropoiesis, as well as for bone morrow suppression.

The regulation of the hematopoietic system is achieved at three levels: 1) at the cellular level of bone marrow stroma, 2) at the humoral level by cytokines, and 3) by catecholamines and other neuroendocrine factors.

Sympathetic nerve endings and bone marrow cells are the main source of bone marrow catecholamines [21,22]. Among the catecholamines, a substantial amount of dopamine was detected in bone marrow [23]. Bone marrow catecholamines originate from sympathetic nerve fibers and from hematopoietic cells directly. Catecholamines of neural origin show a circadian rhythmicity. Adrenoceptors present on bone marrow cells include the 1-subtype, which seems to mediate the catecholaminergic control of hematopoiesis. It has been well documented that there are α- and, β- adrenergic receptors on the surface of the erythrocytes. However, β- receptors are more important in erythropoiesis. Administration of an α- adrenergic stimulant did not elicit the erythropoietic effect, whereas a, β- adrenergic stimulant induced erythropoiesis in an in vitro culture of erythroid progenitor cells [24]. Since, β-adrenergic receptors are abundant on the surface of erythrocytes, the effect of, α- adrenergic blocking agents has often been investigated using erythrocytes [25,26].

In accordance with these findings, in our present study we observed some abnormalities of hematopoiesis after electrolytic lesion of the central dopaminergic neurons from the substantia nigra by means of 6-OHDA. 6-OHDA is a useful chemical agent for inducing neurogenic anemia.

Acknowledgements

This work was supported by a grant from the National Council of Scientific Research and University Education (grant CNCSIS, A type, no. 639/2006), Romania.

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