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

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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.

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Gereç ve Yöntemler: Deneyin başlangıcında 200 ± 50 g ağırlığında 30 adet Wistar sıçan kullanılmıştır. Siyah madde, 0,1% askorbik asit içeren 4 μl serum fizyolojik içinde çözülen ve 4,50 dakikada fazla süreyle Hamilton mikroenjekktörüyle uygulanan 8 mikrogramlik (serbest baz) 6-OHDA stereotaksik mikroenjeksiyon ile sağı tek taraflı olarak lezyonlanmıştır. Nöroşorjurijinden 7 gün sonra, beyaz kan hücrelerinin toplam sayısını (WBC), kirmızı 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, yalnızca operaşyonu tabi tutulmuş sıçanlara karşılaştırıldığında, beyaz kan hücrelerinde (p<0,03), kirmızı kan hücrelerinde (p<0,01) ve hemoglobin seviyesinde (p<0,02) önemli ölçüde azalmaya tetiklemiştir. Bunun aksine, 6-OHDA ile lezyonlanan sıçanlarda eritrosit oranları (ortalama hücre hacmi, MCV (p<0,04); ortalama hemoglobin, MCH (p<0,01)) sahte operaşyonu tabi tutulmuş sıçanlara oranla büyük ölçüde artış göstermiştir.

Sonuç: Bütün olarak bakıldığında, elede edilen veriler merkezi dopaminerjik sistemin eritrosit dinamiklerinin regulasyonunda önemli bir rol oynadığını göstermektedir. (Turk J Hematol 2008; 25: 7140-4)

Anahtar kelimeler: Substantia nigra, 6-OHDA, Hematolojik parametreler.


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 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.
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 × 10⁹/mm³ vs 6.8 ± 0.4 × 10⁹/mm³, U=36.5]
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 hematopoietic system 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. 6-OHDA is a useful chemical agent for inducing neurogenic anemia.

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.

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References


