ABSTRACT
The unilateral or bilateral lesions of the Substantia nigra pars compacta (SNc) by chemical neurotoxins such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are main method for generating the parkinson's animal models. But these neurotoxins are expensive, dangerous, difficult to use and inaccessible conveniently due to the international economical sanctions in Iran. The present study was designed to find a new animal model for Parkinson's disease. Forty eight animals were included in this study and divided into six groups. One group was selected as the control and also two groups as the sham of the SNc lesion. Other groups were subjected to lesion of SNc by 6-OHDA, MPTP and electrical lesion. Using histological studies and Murprogo's method precision, the effectiveness of the electrical lesion was compared with that of chemical lesions. Our results showed the same extent of lesion was provided with electrical insult and neurotoxins. Due to the technical convenience, low cost, accessibility and the same potency of electrical- and neurotoxins-induced lesion, this model can be used as an alternative method for creating the Parkinson's models.

Keywords: 6-hydroxydopamine (6-OHDA), Electrical lesion, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Substantia nigra pars compacta, Parkinson's animal model

MATERIALS AND METHODS:

Animals
Forty eight male albino Wistar rats (200-250g) were used in the present study. The animals were purchased...
from Pasteur Institute of Iran and then housed in groups of ten in stainless steel cages, handled daily, and were provided food and water ad libitum. A 12-h light/12-h dark cycle was maintained, and the animals were tested during the light cycle. The experiments were carried out in accordance with recommendations from the declaration of Helsinki and the internationally-accepted principles in the use of experimental animals. In this study, we divided animals into six groups, each eight rats.

**Drugs and Solvents**

6-OHDA HBr and MPTP were purchased from Sigma (Chemical Co., St. Louis, MO, USA) and ascorbic acid was purchased from Merck (Germany).

**General principles of the Surgery**

Each rat was anesthetized separately by injection of 75 mg/kg ketamine combined with 8 mg/kg Xylazin intraperitoneally. Then, we prepared the rats for surgery and placed them in the stereotaxic apparatus (Stoelting, IL, USA). The left SNc, region of the nigrostriatum was targeted. Stereotaxic coordinators for the left SNc region were set at -4.8 mm posterior and -1.6 mm lateral to bregma and 8.1 mm for 6-OHDA and MPTP or 8.2 mm for electrical lesion ventral to the surface of the skull according to the atlas of Paxinos and Watson. [9]. It should be notified that we used unilateral lesion of SNc in all investigated lesioned groups.

**General procedure of the electrical lesion**

The stainless steel electrode was placed in the left SNc and destroyed the area by Electrical Lesion Maker (Siemens Company, Germany), using electrical current (1mA, 10 seconds). Then, they were kept in individual cages for 7–10 days after the surgery for recovery.

**General procedure of the 6-OHDA lesion**

The skull was exposed and a hole was drilled through the skull in the area overlying the left SNc. A guide-cannula was lowered into the brain and fixed to the cranium with miniature screws and acrylic dental cement and the incision was closed with sutures. Surgery was performed using sterile instruments and aseptic conditions. Then 6-OHDA HBr (10μl of a solution based on 66.7 μg, base form, in ascorbic acid 0.1%) was injected into the left SNc. This procedure has been described in detail previously [10]. Rats were allowed to recover from the surgery for 7–10 days in individual transparent test cages.

**General procedure of the MPTP lesion was as follows:**

The skull was exposed and a hole was drilled through the skull in the area overlying the left SNc. A guide-cannula was lowered into the brain and fixed to the cranium with miniature screws and acrylic dental cement and the incision was closed with sutures. Surgery was performed using sterile instruments and aseptic conditions. Then MPTP (5 μl of a solution based on 2 mg MPTP in sterile 0.9% saline) was injected into the left SNc. This procedure has been described in detail previously [11]. Rats were allowed to recover from the surgery for 7–10 days in individual transparent test cages.

**Rigidity evaluations:**

At the time of study, all animals exhibited rigidity, a loss of vocalisation, diminished blinking, incoordination and a course action tremor. Murprogo’s Method [12] was used to measure the rigidity of all subjected animals at the times: 0, 20, 40, 60, 90, 120, 180 and 240 min. The wood-platforms with the steps of 3 and 9 cm were used in this study. For the procedure of behavior experiments, the animal was put on the bench. when the animal did not move by touch, it received the score of 0.5. Then, the right hand of the animal was placed on the wood-platform with the height of 3 cm. If the animal did not take its hand off the platform after at least 10 seconds, it received the score of 0.5. Rigidity evaluation was repeated for the left hand of the animal as well. In the next stage of the procedure, the right hand of the animal was placed on the wood-platform with the height of 9 cm, so that any other parts of the animal did not touch the platform. The animal was given score of 1, if it did not take its hand off the platform after 10 seconds. Finally, the test was repeated in the same way for the left hand. Each animal that had full rigidity (PD) was given a total score of 3.5. The results of the Murprogo’s test are mentioned in Table 1.

The effects of neurotoxin vehicles on the rigidity or cellular damage have not been investigated because the previous studies have proven the inertness of saline or ascorbic acid in the models (3, 7, 11). Also, unnecessary use of animals is in contrast with internationally-accepted principles in the use of experimental animals.

**Table 1. Mean of rigidity grades as M ± S.E.M in investigated groups (N=8)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>240</th>
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<tr>
<td>Control</td>
<td>0±0</td>
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<td>Sham of electrical lesion</td>
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<tr>
<td>Sham of 6-OHDA and MPTP lesion</td>
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<td>Electrical lesion</td>
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<tr>
<td>6-OHDA lesion</td>
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<td>MPTP lesion</td>
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Electrical Lesion of Substantia Nigra Pars Compacta

Evaluations the electrical method

In vitro investigation of electrical lesion method

Estimation of violence and duration of the lesion was accepted empirically in vitro by determination of clot-dimensions and comparison with the atlas of Paxinos and Watson in electrocardiograph gel caused by electrical maker.

In vivo investigation

The experiments including the Murprogo's method and histological studies were carried out to evaluate and...
compare the electrical lesion with 6-OHDA- and MPTP-induced lesion. After the rigidity test, each suspected animal to PD was decapitated and the brain was removed and kept in a 10% formalin solution. Selected brains were cut on a cryostat as 50 µm thick coronal sections, mounted on glass slides, and stained with H&E. Sections were examined under a light-microscope to find the accuracy of lesion of the left SNc. Finally, the histological results were compared together. The Fig. 1 shows the accuracy and the precision of the lesion.

**RESULTS**

*Effects of different types of the SNc lesion on rigidity*

All of SNc-lesioned rats were given a total score of 3.5 for rigidity test. It is noteworthy that electrical lesion of SNc produced animal model of PD similar to the model produced by 6-OHDA and MPTP.

*Histological comparisons between lesion methods*

Histological studies showed the same potency, precision and accuracy of SNc lesion for electrical lesion in comparison with chemical neurotoxins, as shown in Fig. 1.

**DISCUSSION**

According to the results of this study, electrical lesion of SNc can be employed as an alternative and useful method for creating the PD models with low cost and conveniently-technical procedure because it does not need to use cannula or preparation of dangerous neurotoxins. Also, at this time our country suffers a lot of economical sanctions and access to chemical neurotoxins is not easy. Therefore, the electrical lesion method at this time can solve the neuroscientists’ problems in creating PD model as an alternative method.

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