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Research Report

Behavioral differences in a rotenone-induced hemiparkinsonian rat model developed following intranigral or median forebrain bundle infusion

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Abstract

A mitochondrial complex-I inhibitor, rotenone was unilaterally infused into the substantia nigra pars compacta (SNpc) or median forebrain bundle (MFB) to create hemiparkinsonian animal models and investigated spontaneous and drug-induced stereotypic rotations, as well as certain postural behaviors in Sprague–Dawley rats. Animals infused intranigrally, but not intra-MFB, with rotenone exhibited spontaneous contralateral rotations immediately after recovery from anesthesia. Head position bias and elevated body swing test showed insignificant contralateral bias in animals with nigral damage but a significant ipsilateral bias in MFB-lesioned rats. General motor activity of the animals was reduced in both the groups as indicated by reduced performance on a Plus-Maze. Intranigrally, rotenone-infused animals exhibited progressive ipsilateral rotations when challenged with *d*-amphetamine on the 7th, 14th, 21st, and 28th days or with apomorphine on 9th, 16th, 23rd, and 30th days. However, animals that received rotenone in MFB exhibited ipsilateral or contralateral rotations when challenged respectively with *d*-amphetamine or apomorphine only in the 5th week (28th and 30th days). Stereotaxic administration of rotenone into SNpc or MFB caused a significant loss of dopamine in the ipsilateral striatum (>80% in SNpc; >95% in MFB), when assayed employing an HPLC equipped with electrochemical detector on the 32nd day. Neuronal loss in SNpc was confirmed in coronal sections stained with cresyl violet and revealed extension of lesion towards SN pars reticulata, in SNpc-infused animals. Our results demonstrate that rotenone-induced neurodegeneration is a slow, yet progressive process similar to that in idiopathic Parkinson's disease and unlike that observed in other classical neurotoxin-mediated lesions which are abrupt and developed in few hours to days. Thus, intranigral or intra-MFB infusion of rotenone could be used for producing hemiparkinsonian animal models in rats. These findings further suggest that, while both *d*-amphetamine and apomorphine-induced stereotypic rotations could be used as a valuable behavioral assay procedure to test novel drugs against Parkinson's disease, yet apomorpine-induced contralateral bias in turning is a reliable indicator of specific destruction in nigrostriatal pathway and development of postsynaptic dopamine receptor supersensitivity.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Parkinson's

Keywords: Stereotypic rotational behavior; Complex-I inhibitor; Head position; Elevated body swing test; Cognitive function; Pesticide-induced neurodegeneration; Progressive animal model; Parkinsonism

1. Introduction

Several animal models are available for studying the pathogenesis of Parkinson's disease (PD). Many of the neurotoxic agents that are used to produce animal models of PD are mitochondrial electron transport chain inhibitors, such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl pyridinium ion (MPP^+), rotenone, and paraquat [25,39,52]. Among these models, rotenone-induced experimental PD is the most recent and less studied. Rotenone model produced through the systemic administration recapitulates most of the features of sporadic PD including Lewy body formation

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in the nigral neurons, unlike other models [2,6,9,57]. However, the mechanism of action of this neurotoxin that culminates in the development of many of the features of PD is still under investigation.

Existing literature demonstrates the involvement of mitochondrial energy crisis, microglial activation, oxidative stress, and apoptosis as the major pathways in rotenone's neurotoxic action. Attenuation of rotenone-induced neurotoxicity in rats by the specific neuronal nitric oxide synthase inhibitor 7-nitroindazole implicated nitric oxide as one of the causative factors of neurodegeneration in this model [26]. Rotenone is shown to activate microglia selectively in the brain [56] and to generate reactive oxygen species in the mitochondria [37] leading to neurotoxicity. In human neuroblastoma SH-Sy5y and fibrosarcoma HT-1080 cell lines, rotenone has been shown to induct caspase activation resulting in apoptosis [34,37]. In the brain slices, rotenone has been observed to preferentially damage dopamine neurons [13]. Very recently, rotenone has been shown to produce parkinsonian symptoms in *Drosophila* species [16]. We have observed that acute intracranial administration of rotenone in rats can cause slow degeneration of the striatal dopaminergic pathway, which is progressive, resulting in biochemical lesions that are similar to those seen in idiopathic PD and exhibiting no fatality [51,52].

While there are several detailed behavioral studies available on 6-OHDA models, not many studies exist on this aspect in rodents administered rotenone. Fleming and colleagues have reported decreased locomotor activity and rearing, as well as difficulty in movement initiation and step adjustment after chronic intravenous or subcutaneous rotenone administration [21]. Bilateral median forebrain bundle (MFB) lesioned rat model of rotenone produced catalepsy and reduced motor activity, which was reversed by L-DOPA treatment [3,4]. It has also been reported that apomorphine failed to elicit stereotypic circling behavior in rats with rotenone-induced unilateral nigral lesion [35]. Most of the studies with 6-OHDA employ unilateral striatal, nigral, or MFB lesions to check the rotational asymmetry, body axis bias, head position, and forelimb use [10,29,30,53]. Out of all these targets, the appropriate lesion site, which can mimic human PD, is still not obvious. MFB lesion can cause a total destruction of A9 and A10 dopaminergic areas resulting in an extensive neurodegeneration than is seen in idiopathic PD, whereas SNpc lesions produce a more selective cell loss [47]. While a number of reports exist relating the degree of dopamine (DA) depletion to drug-induced rotational response [22,27,28,31,54], only few studies are available linking rotational bias or stereotypic severity to the target of destruction [36,41]. Moore and colleagues evaluated behavioral deficits after nigral and ventral tegmental area (VTA) lesions [41]. While the loss of nigral neurons correlated with apomorphine-induced rotations, the additional involvement of VTA lesion lessened the magnitude of rotations [41]. In yet another study, *d*-amphetamine induced contralateral rotations in SNpc-lesioned, and ipsilateral rotations in globus pallidus lesioned rats [36].

Administration of DA-releasing agent amphetamine produces a more active striatum contralateral to the side of infusion in a unilaterally lesioned animal and makes it move towards the lesioned side, while apomorphine, a DA agonist, induces contralateral rotations in these animals because of the denervation-induced supersensitivity [27,53,59]. Although drug-induced rotational behavior has been conventionally used for the behavioral analysis of animals lesioned unilaterally, apomorphine-induced rotations usually show up when there is a higher degree of DA depletion, existing for a long duration. We investigated spontaneous behavioral abnormalities too in the present study, and such behavioral tests in a drug-free state may be useful while designing therapeutic strategies. We examined the head position bias and conducted elevated body swing test (EBST) for the analysis of the behavior abnormalities in unilaterally lesioned animal models [30,50]. We assessed the behavior of lesioned animals in a Plus-Maze to study the motor activity by monitoring the number of entries into either of the arms in unit time [49]. All these behavioral parameters are neither analyzed in rotenone model previously nor the comparison between MFB and SN lesions made. Thus, in the present study, for the first time, we report spontaneous behavioral abnormalities as well as DA-agonists-induced differential rotations in unilateral rotenone-lesioned rats.

Animal models of PD with unilateral damage of the nigrostriatal system are suitable for use in the development of neuroprotective and neurotrophic treatment strategies. In this study, we explored the overall motor activity, as well as the drug-induced (DA releaser and agonist) and drug-free (spontaneous rotations, head position bias, EBST) behavioral changes in the rotenone model and compared the effects of SNpc and MFB lesions. Our aim was to find whether rotenone is capable of inducing these behavioral deficits that are usually demonstrated in 6-OHDA lesion models and whether there exists any variation when injury is made in two different locations in the dopaminergic nigrostriatal pathway.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (250–300 g) from the Institute colony were used in the present study. The animals were maintained under standard conditions of 12 h light/dark cycles, 22 ± 1 °C temperature and 60 ± 5% humidity. They were provided food and water ad libitum. The experimental protocols met the National Guidelines on the *Proper Care and Use of Animals in Laboratory Research* (Indian National Science Academy, New Delhi, 2000) and were approved by the Animal Ethics Committee of the Institute.