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Chronic exposure to rotenone, a dopaminergic toxin, results in peripheral neuropathy associated with dopaminergic damage

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HIGHLIGHTS

- Rotenone induced peripheral motor neuropathy and decreased MCV.
- Decrease in MCV might be a viable biomarker for central dopaminergic neuronal damage.
- Correlation between peripheral motor damage and dopaminergic damage by rotenone.

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ABSTRACT

Rotenone, a widely used pesticide, causes a syndrome in rats that replicates, both pathologically and behaviorally, the symptoms of Parkinson's disease (PD). In the present study, we sought to determine if a chronic exposure to rotenone, resulting in dopaminergic loss, could also lead to peripheral neuronal damage related to motor dysfunction. Adult male Sprague-Dawley rats ($n = 14$) were treated with rotenone (1 or 2 mg/kg, s.c., once daily) on days 1, 3, 6, 8, 10, 13, 15, 17, 21, 22, and 27 to minimize mortality. Control rats received vehicle (DMSO) injections. Animals were weighed on the days of injection and monitored daily. A mortality of 21% was observed in rotenone treated rats. The motor nerve conduction velocity (MCV) was assessed using action potentials detected from the tail muscle through surface receiver electrodes installed around the distal portion of the tail. Rats exposed to rotenone often developed hind limb paresis with a significant decrease in MCV as detected in tail nerves ($p < 0.05$). Animals were then sacrificed, either 24 h after rotenone exposure on day 6 or 24 h after the last dose of rotenone on day 27. The striatum and sciatic nerves were dissected on dry ice and flash-frozen and kept at −80 °C until further analysis. Striatal dopamine (DA) was analyzed using HPLC-ECD and sciatic nerve pathology was analyzed for neurodegeneration. A time-dependent rotenone-induced striatal depletion of DA (60% after 7 days and 80% after 27 days) was observed. Furthermore, Neurofilament-neurofilament B, Flouro-Jade C and myelin basic protein analyses suggested a time-dependent rotenone-induced neurodegeneration in sciatic nerves. These data, for the first time, indicate an association between dopaminergic damage and peripheral motor nerve degeneration in an animal model of dopaminergic toxicity. Peripheral motor nerve dysfunction in rats following a chronic exposure to rotenone may serve not only as a relevant experimental model of motor neuropathy but also as a peripheral marker of dopaminergic neuronal damage to the central nervous system.

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1. Introduction

Rotenone is a potent lipophilic inhibitor of complex I of the mitochondrial electron transport chain [1]. Chronic rotenone exposure leads to mitochondrial respiratory chain inhibition and dopaminergic neuronal loss. At low doses, rotenone alters calcium signaling and can induce oxidative stress, apoptosis and α-synuclein aggregation ("Lewy neurites"), which is also a characteristic of early stages of Parkinson's disease [3,13]. Chronic administration of low doses of rotenone induces motor anomalies even in animals that do not develop histological signs of Parkinson's disease [10]. Striatal tyrosine hydroxylase immunoreactivity (TH-IR) was markedly decreased in rats exposed to high doses of rotenone. However, loss of striatal TH-IR was not correlated with motor behavior in individual rats.

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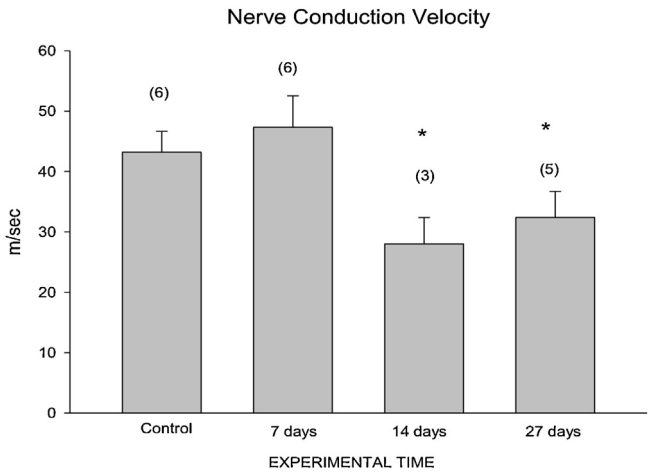

Fig. 1. Motor nerve conduction velocity recorded at 7, 14 and 27 days after exposure to rotenone (1–2 mg/kg, s.c.) in the tail nerve of injections. Mean ± SEM. $^{*}p < 0.05$ when compared to control at day 27. Numbers is parentheses indicate sample size.

Animal models of neuropathy mediated by chemical toxicants are broadly relevant to the area of neurotoxicology. Motor nerve conduction velocity (MCV) testing is frequently used in experimental models of neurotoxicity caused by exposure to aromatic compounds [8] and pesticides [5] or associated with metabolic disorders (e.g., diabetes) neuropathies [7]. In human electro-diagnostic medicine, the testing of motor axons allows one to reliably detect conduction block associated with multifocal demyelination [9]. Rotenone-induced motor neuronal death in the rat spinal cord has been shown, suggesting that spinal cord damage may be linked to the degeneration of higher brain nuclei [12].

The present study evaluated the effect of chronic rotenone exposure on motor nerve conduction and associated peripheral neurotoxic damage. Furthermore, this study also revealed an association between peripheral motor dysfunction induced by chronic rotenone exposure and the well-known dopaminergic neurotoxicity of rotenone.

2. Materials and methods

2.1. Animal treatment

Adult, male Sprague-Dawley rats of the Charles River cesarean delivered (CD) strain 23 were used in the study. Animals were kept under controlled environmental conditions (temperature 22 °C, relative humidity 45–55%, 12-h light/dark cycle) and housed individually with NIH-41 Irradiated Rodent Diet (Harlan Teklad, Madison, WI) chow and tap water supplied ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Center for Toxicological Research (NCTR) and conducted in full accordance with the PHS policy on humane care and use of laboratory animals and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23), revised 1996. Rats were randomly allocated to treatment groups and they were injected subcutaneously (s.c.) with rotenone (1–2 mg/kg once daily) on days 1, 3, 6, 8, 10, 13, 15, 17, 21, 22, and 27 to minimize mortality. Control rats received vehicle (DMSO) injections according to the same schedule and at the same volumes as the rotenone. All animals were weighed on the days of injection and monitored daily.

2.2. Electrophysiology of the tail nerve

The MCV of the caudal tail nerve was measured at 7, 14, and 27 days of the experiment as described earlier [2]. Rats were anesthetized with isoflurane and general anesthesia was maintained

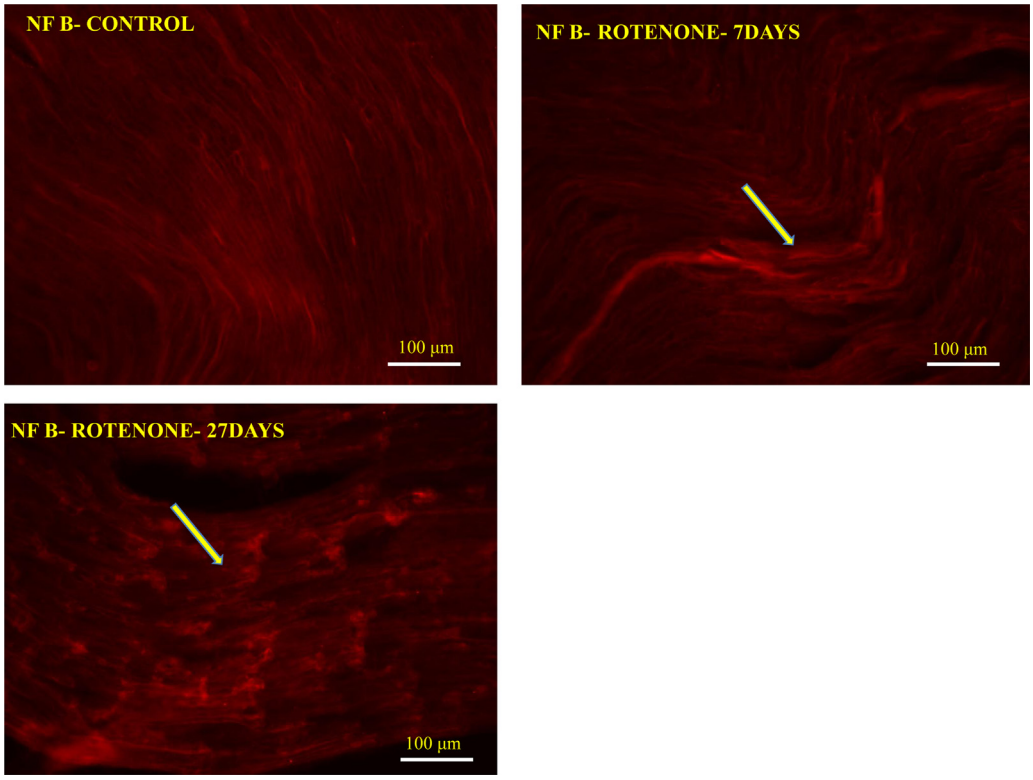

Fig. 2. Representative photomicrographs from histopathological analysis of neurofilament B in control animals and in animals at 7 and 27 days after the start of exposure to rotenone (1–2 mg/kg, s.c.). Intact neurofilament B in controls was observed as compared to progressive denervation and fragmentation of sciatic nerves represented by structural alterations (arrows) in neurofilament B anatomy 7 and 27 days after rotenone exposure.