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Research Report

Intranasally-administered deferoxamine mitigates toxicity of 6-OHDA in a rat model of Parkinson's disease

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ABSTRACT

Deferoxamine (DFO) has shown therapeutic promise for the treatment of Parkinson's disease (PD) as it has reduced both behavioral and biochemical deficits when injected into the brain of rodent models of PD. Intranasally administered DFO targets the brain directly but non-invasively and has been effective in animal models of stroke and Alzheimer's disease. In this study we sought to determine whether intranasal (IN) DFO could be neuroprotective for PD in a rat model. PD was induced with a unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle, while sham surgery rats received saline injections. Rats were pre-treated three times with either IN DFO or saline (starting 4 days before 6-OHDA), and post-treated twice/wk for one month before behavioral tests. In the apomorphine-induced rotational test, IN DFO significantly decreased the number of contralateral turns after injection of apomorphine HCl ($p<0.05$). Also, IN DFO significantly decreased limb asymmetry in the rearing tube as measured with contralateral limb touches ($p<0.05$). The IN DFO treatment yielded a trend towards decreased contralateral foot-slips on the tapered balance beam, though the difference was not significant. Finally, IN DFO-treated rats had increased preservation of tyrosine hydroxylase immunoreactive neurons in the substantia nigra ($p<0.05$). These results confirm that DFO is beneficial in a 6-OHDA model and demonstrate improvement in motor deficits and dopaminergic neuronal survival with non-invasive intranasal delivery, making this an attractive potential treatment for PD.

Abbreviations: DFO, deferoxamine; IN, intranasal; 6-OHDA, 6 hydroxydopamine; PD, Parkinson's disease; AD, Alzheimer's disease; TH IR, tyrosine hydroxylase immunoreactive; PBS, phosphate buffered saline; ICV, intracerebroventricular; LIP, labile iron pool; GSK3β, glycogen synthase kinase 3β; HIF-1α, hypoxia inducible factor-1α; POD, Precision Olfactory delivery; HFA, hydrofluoroalkane; SN, substantia nigra

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1. Introduction

Parkinson's disease (PD) is a neurodegenerative disease that results largely from the death of dopaminergic cells in the substantia nigra (SN), and current treatment options are largely based on compensating for the loss of dopamine in the brain. Another neuropathological trait of PD is a buildup of excess iron in the SN, and thus, metal chelators have been suggested as possible treatment for PD (Youdim et al., 2004; Jellinger, 2013). Deferoxamine (DFO) is an iron chelator that traditionally is used for treatment of iron-overload in human patients, but has also been tested in animal studies to treat PD (Ben-Shachar et al., 1992), Alzheimer's disease (AD) (Percy et al., 2011), and stroke (Selim and Ratan, 2004; Hanson et al., 2009), and can be beneficial when given either before or after disease onset. In a 6-hydroxydopamine (6-OHDA) rat model of PD, systemically delivered DFO alleviated both the build-up of iron in the brain and behavioral problems related to the disease (Youdim et al., 2004; Dexter et al., 2011). Another animal study delivered DFO along with quercetin, a bioflavonoid, via intraperitoneal injections and found similar results (Haleagrahara et al., 2013). While these studies demonstrated that DFO could be used as a treatment for animal models of PD, most used some form of systemic administration, or intracranial injections that are not suitable for human use.

DFO has been used with intranasal (IN) delivery to treat rodent models of neurodegenerative disease and holds potential as a clinically relevant option for the treatment of PD (Hanson and Frey, 2008). Intranasal delivery allows for treatments to be targeted to the brain through application to the nasal cavity, and is beneficial for several reasons. Primarily, IN delivery allows drugs to bypass the blood-brain barrier and target the CNS directly via the olfactory and trigeminal nerves (Hanson and Frey, 2008; Dhuria et al., 2010), while minimizing unwanted systemic side-effects (Pires et al., 2009). Intranasal delivery of drugs has been used in both animals models and humans (Born et al., 2002; Hanson et al., 2009; Marks et al., 2009; Dhuria et al., 2010). Direct brain targeting of DFO via IN administration is especially beneficial for DFO because DFO has a short half-life in blood (Summers et al., 1979; Allain et al., 1987). Intranasal DFO has been tested in mouse and rat models of stroke, AD, and PD (Hanson et al., 2009; Fine et al., 2012; Febbraro et al., 2013). Recently, Febbraro et al. (2013) used an α-synuclein rAAV model of PD and treated awake rats intranasally with DFO. They found that DFO improved behavioral deficits as well as prevented an increase in Fe^{3+} positive cells (Febbraro et al., 2013).

In the current study, we determined whether IN DFO would alleviate behavioral and biochemical symptoms in a 6-OHDA model of PD in rats. The 6-OHDA destroys dopaminergic neurons, thus mimicking PD (Duty and Jenner, 2011). Rats underwent stereotaxic surgery and were given a unilateral injection of either 6-OHDA or saline to the right side of the brain in the medial forebrain bundle. The rats were treated with either IN DFO or PBS using a Precision Olfactory Delivery (POD) device (Impel Neuropharma, Seattle, WA), which targets drugs to the upper third of the nasal cavity using hydrofluoroalkane (HFA) gas. The DFO-treated groups of rats were given IN DFO both before and after administration of 6-OHDA, as disease onset occurs immediately upon administration of 6-OHDA rather than as a progressive neurodegenerative disorder, which is a weakness of the model (Duty and Jenner, 2011). Behavior tests included the apomorphine-induced rotational test, rearing tube, tapered balance beam, and open field test. Rats were then euthanized and brain tissues were examined. We found that IN DFO improved behavior in both the apomorphine-induced rotational test and rearing tube test, and indicated improvement in the balance beam test. Also, the tissue analysis showed that IN DFO protected against the loss of dopaminergic neurons in the lesioned area. These results indicate that IN DFO is a potential method of protecting against and treating Parkinson's disease.

2. Results

2.1. General health

There were no obvious health problems or mortality for any rats during the study. There was no significant difference in

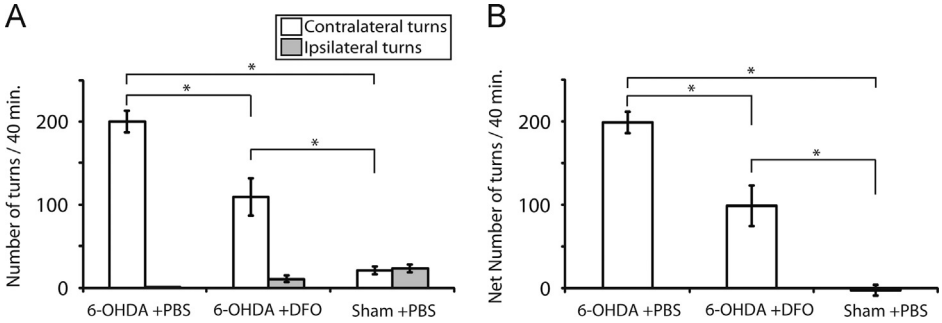

Fig. 1 – Apomorphine-induced rotational test data for (A) number of turns and (B) net number of turns (contralateral–ipsilateral). Long Evans rats were dosed with IN DFO or PBS for 5 weeks (n=6–11 rats/group). Each rat was given a subcutaneuous injection of apomorphine HCl (Sigma-H116) (0.4 mg/kg) and observed for 40 min. 6-OHDA+PBS displayed significantly more total turns than Sham+PBS for both measures (*$p<0.05$). 6-OHDA+DFO had less turns than 6-OHDA+PBS for both measures (*$p<0.05$). Error bars are SEM. 6-OHDA=6 hydroxydopamine, PBS=phosphate-buffered saline, IN=intranasal, DFO=deferoxamine, and SEM=standard error of the mean.