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A novel dual GLP-1 and GIP receptor agonist is neuroprotective in the MPTP mouse model of Parkinson's disease by increasing expression of BNDF

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ABSTRACT

The incretins glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are growth factors with neuroprotective properties. GLP-1 mimetics are on the market as treatments for type 2 diabetes and are well tolerated. Both GLP-1 and GIP mimetics have shown neuroprotective properties in animal models of Parkinson's and Alzheimer's disease. In addition, the GLP-1 mimetic exendin-4 has shown protective effects in a clinical trial in Parkinson's disease (PD) patients. Novel GLP-1/GIP dual-agonist peptides have been developed and are tested in diabetic patients. Here we demonstrate the neuroprotective effects of a novel dual agonist (DA-JC1) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. MPTP was injected once-daily (20 mg/kg i.p.) for 7 days, and the dual agonist was injected 30 min later i.p. (50 nmol/kg bw). The PI3k inhibitor LY294002 (0.6 mg/kg i.v.) was co-injected in one group. DA-JC1 reduced or reversed most of the MPTP induced motor impairments in the rotarod and in a muscle strength test. The number of tyrosine hydroxylase (TH) positive neurons in the substantia nigra (SN) was reduced by MPTP and increased by DA-JC1. The ratio of anti-inflammatory Bcl-2 to pro-inflammatory BAX as well as the activation of the growth factor kinase Akt was reduced by MPTP and reversed by DA-JC1. The PI3k inhibitor had only limited effect on the DA-JC1 drug effect. Importantly, levels of the neuroprotective brain derived neurotropic factor (BDNF) were reduced by MPTP and enhanced by DA-JC1. The results demonstrate that DA-JC1 shows promise as a novel treatment for PD.

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1. Introduction

Parkinson disease (PD) is the second most common neurodegenerative disease after Alzheimer disease, and current demographic trends indicate a life-time risk approaching 4% and predict a doubling of prevalence by 2030 (Schapira, 2013). It is characterized clinically by a variety of motor dysfunctions such as resting tremor, bradykinesia, rigidity and postural instability (Langston, 2002). These symptoms are attributed to the reduction in striatal dopamine (DA) level, which results from the selective and progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Moore et al., 2005; Wakamatsu et al., 2008). Several risk factors have been identified, and type 2 diabetes is one of these (Hu et al., 2007; Schernhammer et al., 2011; Sun et al., 2012; Wahlqvist et al., 2012). Previous studies have documented the importance of insulin signalling in the brain (Freiherr et al., 2013; Ghasemi et al., 2013; van der Heide et al., 2006), and the fact that insulin signalling is compromised in the brains of patients with PD (Aviles-Olmos et al., 2013b; Moroo et al., 1994; Morris et al., 2011). In diabetes, analogues of incretin hormones have been developed to improve insulin signalling (Campbell and Drucker, 2013; Holst, 2004). The key incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Baggio and Drucker, 2007; Campbell and Drucker, 2013). It has been confirmed that GLP-1 receptor agonists and GIP receptor agonists can pass through the blood–brain barrier (Faivre and Holscher, 2013b; Hunter and Holscher, 2012; McClean and Holscher, 2014), protect neurons under oxidative stress, inhibit apoptosis, promoting neuronal proliferation and neuronal cells to grow new projections (Holscher, 2014b; Ji et al., in press; Li et al., 2010b, 2015; Sharma et al., 2013). GLP-1 receptor agonists have shown protective effects in animal models of Alzheimer's disease (Bomfim et al., 2012; Li et al., 2010a; McClean et al., 2011), and clinical trials have started (Holscher, 2014a) with first positive results having been published (Gejl et al., 2015). GIP analogues also have shown protective effects in animal models of Alzheimer's disease (Duffy and Holscher, 2013; Faivre and Holscher, 2013a, 2013b). Importantly, previous investigations found that GLP-1 receptor agonists also showed good neuroprotective effects in animal models of PD (Bertilsson et al., 2008; Harkavyi et al., 2008; Li et al., 2009; Liu et al., 2015; Zhang et al., 2015) and showed good effects in a pilot study in PD patients (Aviles-Olmos et al., 2013a, 2014).

The new drug DA-JC1, which is a dual-GLP-1/GIP receptor agonist (see Section 4 for the peptide sequence), shows superior effects in animal models of diabetes compared with liraglutide. This dual incretin agonist has been engineered to activate both GLP-1 and GIP receptors with comparable affinity, and demonstrated enhanced insulinotropic efficacy relative to single GLP-1 agonists (Finan et al., 2013). Some of these dual agonist peptides are already in clinical trials in patients with diabetes, and first results show good effects with fewer side effects compared to GLP-1 mimetics (Finan et al., 2013). We therefore tested the effects of a potent GLP-1/GIP receptor agonists in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropypridine (MPTP) mouse model of PD. MPTP can selectively damage neurons in the nigrostriatal dopaminergic pathway and cause Parkinsonism in humans, nonhuman primates, and mice, mice have therefore become well accepted as a model for PD (Bove and Perier, 2012; Glover et al., 1986; Morin et al., 2014).

In a previous study, we have confirmed that the DA-JC1 has neuroprotective effects in MPTP-induced mice by increasing the number of TH, reducing the activation of astroglia and microglia (Cao et al.,submitted for publication). To further analyse the drug effects on neurodegenerative biomarkers in the brain of c57bl mice, we analysed the expression levels of brain derived neurotrophic factor (BDNF), a key neuroprotective growth factor (Kuipers and Bramham, 2006; Nagahara et al., 2013), and the expression of apoptosis signalling proteins (BAX, Bcl-2) (Sharma et al., 2013) using immunohistochemical and western blot methods. Moreover, we sought to determine whether neuroprotection by DA-JC1 against MPTP is mediated by the activation of the PI3K/Akt pathway, a key growth factor second messenger pathway (Holscher, 2014b; Talbot et al., 2012).

2. Results

2.1. DA-JC1 improved the MPTP-induced impairments in motor coordination and in muscle strength

A One-way ANOVA found an overall difference between groups for Rotarod performance: ($F=38.7$; $P<0.001$) for muscle strength test: ($F=10.9$; $P<0.001$), followed by Fisher's Least Significant Difference test (LSD) post-hoc tests. DA-JC1 enhanced motor coordination of MPTP-treated animals as reflected in the time they were able to stay on the RotaRod and improved their muscle strength as see in a traction test ($P<0.05$). Animals that had received treatment with MPTP showed significant impairments in motor coordination compared to the control animals that had received saline ($P<0.05$). Treatment of DA-JC1 significantly reversed the motor impairments induced by MPTP ($P<0.05$). However, no significant difference was found between the MPTP+DA-JC1 and MPTP+DA-JC1+LY294002 groups ($P>0.05$). Data are represented as mean ± SEM, $n=10$ per group (see Fig. 1).

2.2. Immunohistochemistry

2.2.1. DA-JC1 attenuated the loss of nigral TH-positive neurons induced by MPTP in the substantia nigra

The protective effects of DA-JC1 on the dopaminergic neurons in the SN of mice treated with MPTP are shown in Fig. 2. A One-way ANOVA showed an overall difference between groups ($F=30.34$; $P<0.001$) followed by LSD-t post-hoc tests. There were significant reductions in the number of TH-positive cells in the SNpc for the MPTP group compared with the control group (36.83 ± 4.62, $P<0.05$). With the treatment of DA-JC1, the number of TH-positive cells was significantly higher than those in MPTP treated mice (48.50 ± 5.32, $P<0.05$). Co-injecting the PI3k inhibitor LY294002 only reduced the DA-JC1 effect marginally (44.6 ± 5, $P<0.05$ vs. controls) and did not reduce the neuroprotective effect of DA-JC1 on neurons in the