Neuroscience 303 (2015) 42–50

NEUROPROTECTIVE EFFECTS OF LIXISENATIDE AND LIRAGLUTIDE IN THE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE MOUSE MODEL OF PARKINSON'S DISEASE

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Abstract—Glucagon-like peptide 1 (GLP-1) is a growth factor. GLP-1 mimetics are on the market as treatments for type 2 diabetes and are well tolerated. These drugs have shown neuroprotective properties in animal models of neurodegenerative disorders. In addition, the GLP-1 mimetic exendin-4 has shown protective effects in animal models of Parkinson's disease (PD), and a clinical trial in PD patients showed promising first results. Liraglutide and lixisenatide are two newer GLP-1 mimetics which have a longer biological half-life than exendin-4. We previously showed that these drugs have neuroprotective properties in an animal model of Alzheimer's disease. Here we demonstrate the neuroprotective effects 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 drugs were injected once-daily for 14 days i.p. When comparing exendin-4 (10 nmol/kg), liraglutide (25 nmol/kg) and lixisenatide (10 nmol/kg), it was found that exendin-4 showed no protective effects at the dose chosen. Both liraglutide and lixisenatide showed effects in preventing the MPTP-induced motor impairment (Rotarod, open-field locomotion, catalepsy test), reduction in tyrosine hydroxylase (TH) levels (dopamine synthesis) in the substantia nigra and basal ganglia, a reduction of the pro-apoptotic signaling molecule BAX and an increase in the anti-apoptotic signaling molecule B-cell lymphoma-2. The results demonstrate that in this study, both liraglutide and lixisenatide are superior to exendin-4, and both drugs show promise as a novel treatment of PD.

Key words: neurodegeneration, growth factor, apoptosis, insulin, incretin, basal ganglia.

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Abbreviations: ANOVA, analysis of variance; BBB, blood–brain barrier; GDNF, glia-derived neurotrophic factor; GLP-1, glucagon-like peptide 1; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; TH, tyrosine hydroxylase.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.054
0306-4522/

INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disorder of motor control, characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the degeneration of projection axons and dopaminergic synapses to the striatum, which leads to tremors, muscular rigidity, bradykinesia, and postural and gait abnormalities (Langston, 2002). There is currently no treatment for this condition. Recently, the neuroprotective and restorative effect of growth factors such as glia-derived neurotrophic factor (GDNF) in cell culture and animal models of Parkinson's disease has drawn attention to the potential of using growth factors as a treatment (Mickiewicz and Kordower, 2011). One major stumbling block for this strategy is the fact GDNF and other growth factors do not cross the blood–brain barrier (BBB) (Holscher, 2014b). The growth factor glucagon-like peptide 1 (GLP-1) and its analogs have shown neuroprotective effects in several disease models of neurodegeneration (Perry and Greig, 2004; Holscher, 2013). Several GLP-1 receptor agonists have been developed as treatments for type 2 diabetes, and some of these can cross the BBB (Kastin et al., 2002; Kastin and Akerstrom, 2003; McClean et al., 2011; Hunter and Holscher, 2012). Previous investigations found that the GLP-1 receptor agonist exendin-4 showed good neuroprotective effects in animal models of PD (Bertilsson et al., 2008; Harkavyi et al., 2008; Kim et al., 2009; Li et al., 2009). Exendin-4 also had been tested in a pilot clinical trial in people with PD and showed encouraging effects (Aviles-Olmos et al., 2013; Aviles-Olmos et al., 2014). Exendin-4 is superior to endogenous GLP-1 as it is resistant to cleavage by the protease DPP-IV and has a much enhanced biological half-life in the blood (Baggio and Drucker, 2007). Newer GLP-1 mimetics have been developed since, and they have longer survival times in the blood stream (Tan and Bloom, 2013). Liraglutide is an acetylated form of the GLP-1 peptide that has a much enhanced half-life and that is on the market as a drug treatment for diabetes (Victoza) (Raun et al., 2007). Lixisenatide also is a novel GLP-1 mimetic that recently has been approved in Europe as a treatment for diabetes (Lyxumia) (Elkinson and Keating, 2013). Both drugs have shown neuroprotective effects in animal models of Alzheimer's disease (McClean et al., 2011; McClean and Holscher, 2014b). We therefore tested the effects of exendin-4, liraglutide and lixisenatide in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

mouse model of PD at doses that showed effect in previous in vivo studies for comparison. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is a neurotoxin precursor to 1-methyl-4-phenylpyridinium (MPP+), which induces classic symptoms of Parkinson's disease by impairing or destroying dopaminergic neurons in the substantia nigra (Nakamura and Vincent, 1986; Gerlach et al., 1991). MPTP is a widely used chemical to induce a Parkinson-like state in animals (Nakamura and Vincent, 1986; Kopin and Markey, 1988; Kim et al., 2009; Li et al., 2009).

EXPERIMENTAL PROCEDURES

Animals

C57Bl6 male mice, 8 weeks old, 25–30 g in weight were randomly divided into 8 groups: (1) control group, (2) MPTP/vehicle group (3) exendin-4 group (4) MPTP/exendin-4 group, (5) liraglutide group, (6) MPTP/liraglutide group, (7) lixisenatide group, (8) MPTP/lixisenatide group. $N = 12$ animals per group). Mice received 20 mg/kg/day MPTP in normal saline intraperitoneally for 7 consecutive days. Drugs dissolved in saline were administered after each MPTP injection. After the 7 days, mice received a further 7 days of drug or saline injection once-daily (Fig. 1). Drug concentrations were exendin-4: 10 nmol/kg i.p., lixisenatide: 10 nmol/kg i.p., liraglutide: 25 nmol/kg i.p. Animals were handled for one week and exposed to the experimental room and the apparatus 2–3 times before commencement of experiments. All experiments were licensed by the UK Home Office (PPL 70 8236).

Peptides and chemicals

The peptides were synthetized by ChinaPeptides Co., Ltd (Shanghai, China) to 95% purity. The purity of the peptide was confirmed by reversed-phase HPLC and characterized using matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry. MPTP, paraformaldehyde (PFA) was purchased from Sigma–Aldrich (St Louis, MO, USA). Rabbit anti- tyrosine hydroxylase (TH), B-cell lymphoma-2 (Bcl-2), BAX, Goat Anti-rabbit IgG H&L (HRP) secondary antibody were obtained from Abcam (Cambridge, UK). The Bicinchoninic acid (BCA) protein assay kit was purchased from Applygen Technologies Inc. (Beijing, China). Sodium chloride, ethylene glycol, and 3,3-diaminobenzidine (DAB) were purchased from ZSGB-BIO Co. (Beijing, China).

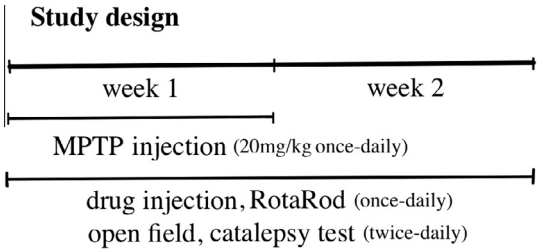

Fig. 1. Study design and timelines.

The transfer polyvinylidene difluoride membranes and Amersham ECL Prime western blotting detection reagent were purchased from GE Life Sciences (USA).

Open-field motor activity test

The open field is a square box (45 cm × 45 cm) with 40 cm high walls that prevent escape. The floor is marked with a grid of lines (separated by 9 cm), The mice were individually placed in one corner of the open field. The number of crossed lines during 10 min of testing session was recorded as the total distance. Animals were tracked by a computerized video system that measured path length (Biosignals, USA).

Rotarod

Mice were placed on a Rotarod system (II-755, IITC Life Science, USA) that accelerated from 5 to 20 rpm over a period of 50s. The length of time that each animal was able to stay on the rod was recorded as the latency to fall, registered automatically by a trip switch under the floor of each rotating drum. A maximum trial length of 180 s was given.

Catalepsy bar test

Catalepsy was evaluated in the bar test immediately after MPTP drug treatment and on every second day after the drug treatment. The frame was made of wood (25 cm long; 5 cm wide, 10 cm high) with a horizontal bar (0.8 cm diameter, 20 cm long) suspended 30 cm above the floor. Catalepsy was evaluated by measuring the mean time taken for a mouse to climb over the bar after being laid across it with its hind limbs on the floor.

Histology

After the experiments, animals were perfused transcardially with PBS buffer followed by ice-cold 4% paraformaldehyde in PBS. Brains were removed and fixed in 4% paraformaldehyde for at least 24 h before being transferred to 30% sucrose solution overnight. Brains were then snap frozen and coronal sections of 30-micron thickness were cut at a Leica cryostat. Sections were taken for the basal ganglia from Bregma 1.54 mm to Bregma −0.34 mm and for the Substantia nigra from −2.46 mm to Bregma −3.88-mm Sections were chosen according to stereological rules (Bondolfi et al., 2002) with the first section taken at random and every 3th section afterward. Between 3 and 6 sections were analyzed per brain. The sections were analyzed on an Axio Scope 1 (Zeiss, Germany) and photographed with a digital camera (AxioCam). A dissector was applied to the images in random orientation to avoid sampling bias (Bondolfi et al., 2002). In the SN, TH-positive cells were counted per dissector. In the other immunohistological assessments, the greyscale (optical density) of stained area per dissector was analyzed using the image analysis program Image J 1.41o with the Multi threshold plug (NIH, USA) (http://rsb.info.nih.gov/ij) (McClean et al., 2011). The scale of staining correlates with the stained antigen.