Neuropeptides xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Neuroprotective effects of the novel GLP-1 long acting analogue semaglutide in the MPTP Parkinson's disease mouse model

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ARTICLE INFO

Keywords:
Incretin
Insulin
Autophagy
Inflammation
Dopamine
Growth factor

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disease, and there is no recognised therapy to cure it. Recently, it has been shown that treatments to improve insulin resistance in type 2 diabetes (T2DM) may be useful for PD patients. In previous studies, the glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide showed good neuroprotective effects in animal models of PD. In addition, the GLP-1 mimetic exendin-4 has shown good protective effects in PD patients in a phase II clinical trial. Here, we report the protective effects of semaglutide (25 nmol/kg ip. once-daily for 7 days), a new long-acting GLP-1 analogue, in the MPTP mouse model of PD. Moreover, we compared the neuroprotective effect of semaglutide with liraglutide given at the same dose. Our work shows that both semaglutide and liraglutide improved 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced motor impairments. In addition, both GLP-1 analogues rescued the decrease of tyrosine hydroxylase (TH) levels, alleviated the inflammation response, reduced lipid peroxidation, inhibited the apoptosis pathway, and also increased autophagy- related protein expression, to protect dopaminergic neurons in the substantia nigra and striatum. Moreover, the long-acting GLP-1 analogue semaglutide was superior to liraglutide in most parameters measured in this study. Our results demonstrate that the new long-acting GLP-1 analogue semaglutide may be a promising treatment for PD.

1. Introduction

Parkinson disease (PD) is a neurodegenerative chronic disorder. With the increase of life expectancy in the industrialized nations, PD patient numbers have increased significantly (Schapira, 2013). The pathology of PD is characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the degeneration of nerve fibers which project to the striatum. This leads to tremors, muscular rigidity, bradykinesia, and postural and gait abnormalities (Kowal et al., 2013). Previous studies have shown that Type 2 diabetes (T2DM) is a risk factor for PD (Gang et al., 2007; Schernhammer et al., 2011; Yu et al., 2012; Wahlqvist et al., 2012). Insulin desensitization may be one mechanism that underlies PD disease progression. Clinical data demonstrate that around 8–30% of PD patients are diabetic, a significantly higher percentage compared to the age matched control group (Gang et al., 2007; Schernhammer et al., 2011; Cereda et al., 2011; Miyake et al., 2010). Previous studies have documented the importance of insulin signalling in the brain (Freiherr et al., 2013; Ghasemi et al., 2013; Lp et al., 2006), and proved that insulin signalling is compromised in the brains of PD patients (Aviles et al., 2013; Moroo et al., 1994; Morris et al., 2011). Analogues of incretin hormones have been developed to improve insulin signalling in T2DM (Campbell and Drucker, 2013; Holst, 2004). These drugs enhance insulin release and insulin sensitivity. The incretin hormone family includes glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Campbell and Drucker, 2013; Baggio and Drucker, 2007; Doyle and Egan, 2003). Importantly, some investigations show that GLP-1 receptor agonists have good neuroprotective effects in animal models of PD (Bertilsson et al., 2010; Harkavyi et al., 2008; Li et al., 2009; Liu et al., 2015a; Zhang et al., 2015). The first GLP-1 mimetic exendin-4 (Exenatide, Byetta, Bydureon) that is on the market to treat T2DM showed a therapeutic effect in preclinical tests (Harkavyi et al., 2008; Li et al., 2009; Kim et al., 2009), and a pilot clinical trial in PD patients (NCT01174810) has shown good effects (Aviles et al., 2013; Iciar et al., 2014; Aviles-Olmos et al., 2014). A follow-up phase II clinical trial confirmed the protective effects in PD patients (Athauda et al., 2017). Liraglutide (Victoza®) is a modified analogue of human GLP-1 (Cho et al., 2014; Manandhar and Ahn, 2015;

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https://doi.org/10.1016/j.npep.2018.07.003
Received 15 April 2018; Received in revised form 9 July 2018; Accepted 9 July 2018

Vilsboll, 2009). Liraglutide and other GLP-1 mimetics showed good protective effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD (Liu et al., 2015a), and liraglutide is currently being tested in a phase II trial in PD patients (clinical trial identifier NCT02953665). The GLP-1 analog liraglutide has a half-life of approximately 12 h in pigs as well as in humans (Holst, 2004; Tan and Bloom, 2013), which requires a once-daily dosing in diabetic patients (Nauck et al., 2009). Semaglutide (Ozempic) is a modification of liraglutide that is protease-resistant by changing the amino acid at position 8, and is being marketed as a new once-weekly drug to treat type II diabetes. It has been approved in the USA, Canada and the EU as a treatment for diabetes (Dhillon, 2018; Hedrington et al., 2018). Semaglutide significantly reduced HbA1c and reduced bodyweight. Semaglutide showed a similar safety profile to the other GLP-1 receptor agonists currently used for the management of T2DM. (Lovshin, 2017). In this study, we investigated for the first time the neuroprotective effects of the once-weekly GIP-1 analogue semaglutide and compare it at equal doses with liraglutide in the MPTP mouse model of PD.

2. Materials and methods

2.1. Chemicals and peptides

Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was purchased from Sigma-Aldrich (St Louis, MO, USA). Other chemicals used were of the highest quality commercially available. Semaglutide and liraglutide (Peptide Purity: 95.77%) was purchased from Synpeptide Co. (Shanghai, China).

The amino acid sequence for GLP-1 (human)

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH2

The amino acid sequence of liraglutide:

NH2-HAEGTFTSDVSSYLEGQAAK[(γE)-(Pal)]EFIAWLVR-GRG-COOH

Pal = palmitoyl acid;

The amino acid sequence of semaglutide: NH2-HXEGTFTSDVSSYLEGQAAK[(γE)-(Pal)]EFIAWLVRGRG-COOH

Pal = palmitoyl acid;

X = aminoisobutyric acid;

2.2. Animals and drug treatments

Male C57BL/6 mice 10 weeks old (20–25 g) were provided by the Experimental Animal Center, ShanXi Medical University. The animals were maintained on 12:12-h light:dark cycle at 24°C ± 1°C and 55 ± 5% humidity with food and water ad libitum. Mice were randomized divided into six groups (n = 12 per group) (i) control group treated with saline alone; (ii) liraglutide group treated with saline and liraglutide (25 nmol/kg ip. once daily for 7 days); (iii) semaglutide group treated with saline and semaglutide (25 nmol/kg ip. once daily for 7 days), (iv) MPTP group treated with MPTP alone (once daily 20 mg/kg ip. for 7 days); (v) MPTP (once daily 20 mg/kg ip. for 7 days) followed immediately by liraglutide treated group (25 nmol/kg ip. once daily for 7 days). (vi) MPTP (20 mg/kg ip. once daily for 7 days) followed immediately by semaglutide treated group (25 nmol/kg ip. once daily for 7 days). At the end of drug treatments, measure the behavioral changes, neuronal damage, inflammatory markers, and other biomarkers.

2.3. Behavioral assessment

2.3.1. Open-field test

The open-field test is used to evaluate locomotor and exploratory activity of PD mice and was conducted on the 8th day after MPTP treatment. The open-field apparatus consisted of a circular arena (d = 35 cm), and a computer tracking system which includes a camera and a computer. Each mouse was placed in the center of the apparatus, and tracking started immediately after. After acclimatizing for 10 min, the distance travelled by the animal was recorded by the tracking system. Each mouse was given one run per day. The apparatus was then cleaned with 75% alcohol and dried between each trial.

2.3.2. Rotarod performance

The rotarod test is to measure motor coordination in the mouse model of PD. The rotarod equipment (YLS-4C, Academy of medical sciences in Shandong, China) consisted of a rotating spindle and five individual compartments that were able to simultaneously test five mice. Three days before MPTP injection, animals were allowed to train on the rotarod every day until the mice learn how to keep balance on it. On the 8th day of the experiment, mice were placed on a rod that accelerated from 0 to 20 rpm over a period of 50 s. The length of time that each animal was able to stay on the rod was recorded as the latency period to fall. The experiment was repeated three times for each animal at 10 min rest intervals to prevent stress and fatigue and a maximum trial length of 180 s pretrial.

2.3.3. Footprint test

Footprint studies were conducted as previously described (Barlow et al., 1996). Briefly, the animals were trained the day before the test in a dark tunnel (10 × 10 × 50 cm). To carry out the test, the forelimbs were dipped in blue ink and the hind limbs in red ink, and the mice were placed at the entry of a dark tunnel (10 × 10 × 50 cm). The footprints were recorded on a clean sheet of white paper placed on the floor of the tunnel. The two initial steps were excluded from the measurements, and only steps performed in a straight line were recorded. To avoid differences in the stride length as a result of velocity variations, the footprints were only recorded when the mice walked along the tunnel with a regular velocity, excluding the mice that performed the test with perceptible velocity alterations. Stride lengths were determined by measuring the distance between each step on the same side of the body. The length of the shortest stride was subtracted from the length of the longest stride to determine the stride variability.

2.4. Brain tissue preparation

All animals were killed on the 9th day after MPTP injection. After Ethyl carbamate anesthetization, the brains of 6 mice per group were selected and substantia nigra and striatum was dissected and immediately frozen at −80°C for immunoblot analysis. Another 6 mice per group were intracardially perfused with 20 ml saline and then fixed with 20 ml of cold 4% para-formaldehyde (PFA). Brains were immediately removed and post-fixed in 4% PFA 24 h for immunohistochemistry analysis.

2.5. Immunohistochemistry

The fixed brain tissue samples were embedded in paraffin, and sections were cut at 4 μm with a semiautomatic microtome (Leica, Wetzlar, Germany). The sections encompassing the SNpc and the striatum were placed on glass slides, then the paraffin was removed from the tissue sections with xylene, and the sections were rehydrated in gradient series of ethanol solutions. The sections were put into H_2O_2 (3%) for 10 min to block the activity of endogenous peroxidase. Antigen retrieval was performed by heating in 10 mmol/L citrate buffer for 10 min. After blocked with 5% BSA, sections were incubated with the primary antibody for TH (rabbit anti-TH; 1:200; Abcam, Cambridge, UK), GFAP (rabbit anti-GFAP; 1:200; Boster Biotechnology Co., Ltd. Wuhan, China) and IBA1 (goat anti-IBA1; 1:200; Boster Biotechnology Co., Ltd. Wuhan, China), 4-HNE (rabbit anti-4-HNE; 1:400; Biosynthesis Biotechnology Co., Ltd. Beijing, China), Bcl-2 (1:400; Bioworld Technology, lnc. MN, USA), Bax (1:400; Bioworld Technology, lnc. MN, USA) at 37 °C for 1 h. Then they were rinsed in PBS and incubated a secondary peroxidase-conjugated antibody kit (Boster, Wuhan, China)