Post-treatment with the GLP-1 analogue liraglutide alleviate chronic inflammation and mitochondrial stress induced by Status epilepticus

Rui-Fang Wang, Guo-Fang Xue, Christian Hölscher, Miao-Jing Tian, Peng Feng, Ji-Ying Zheng, Dong-Fang Li.

ARTICLE INFO

Keywords:
Liraglutide
GLP-1
Apoptosis
Inflammation
Neuroprotection
Insulin

ABSTRACT

Glucagon-like peptide-1(GLP-1) is a growth factor that has neuroprotective and anti-inflammatory properties. The protease resistant GLP-1 analogue liraglutide has been shown to be neuroprotective in previous studies in animal models of Alzheimer’s disease or Parkinson’s disease. Status epilepticus (SE) is a complex disorder, involving many underlying pathological processes, including excitotoxic and chronic inflammatory events. The present pilot study aims to investigate whether liraglutide alleviates the chronic inflammation response and mitochondrial stress induced by SE in the lithium-pilocarpine animal model. We found that treatment with 25nmol/kg. i.p. once-daily after the induction of SE for 7 days reduced chronic inflammation as shown by reduced numbers of activated microglia and astrocytes, and reduced levels of TNF-α and IL-1β in the hippocampus. The mitochondrial stress marker BAX was reduced and the survival factor Bcl-2 was enhanced by liraglutide. Blood glucose levels were not affected by liraglutide. We show for the first time that liraglutide can reduce the chronic inflammation and mitochondrial stress induced by SE, and the results suggest that GLP-1 receptor agonists such as liraglutide have restorative and protective effects in the brain after SE and could serve as a potential treatment.

1. Introduction

Status epilepticus (SE) is a neurological condition that leads to substantial morbidity and mortality (Fountain, 2000; Neligan and Shorvon, 2011). On average, about 1/3-1/2 SE patients will get worse, and about 2/5 refractory epilepsy patients will die (Chen et al., 2011; Holtkamp, 2005; Vooturi et al., 2014). Surviving patients may suffer from cognitive disorders or neuronal impairments (Kantane et al., 2017; Hesdorffer et al., 1998; Ambrogini et al., 2014). At present, benzodiazepines such as lorcazepam and diazepam are widely applied as first-line treatments for SE (Trandafir et al., 2015). Although injections of diazepam (10 mg/kg) after the first seizure can effectively stop seizures and reduce mortality, it fails to impact on the resulting neurodegeneration (Pouliot et al., 2013; Pitkanen et al., 2005). The currently available antiepileptic drugs can only alleviate symptoms of seizure, but not reduce the neurodegenerative processes induced by SE.

Chronic inflammation of the brain also plays a crucial role in epileptogenesis. Inflammation of the hippocampus involves activated glial cells and the release of specific inflammatory cytokines (Ravizza et al., 2011; Vezzani et al., 2013). Activated microglia and astrocytes secrete pro-inflammatory cytokines such as interleukin 1β (IL-1β), tumour necrosis factor-α (TNF-α) and chemoattractant (MCP1) which can decrease seizure threshold (Vezzani et al., 2013; Vezzani et al., 2008; Puttachary et al., 2015; Vezzani et al., 2011). In addition, peripheral immune cells including leukocytes, monocytes/macrophages and granulocytes infiltrate the CNS after SE which contribute to the chronic epilepsy (Fabene et al., 2008; Varvel et al., 2016; Vinet et al., 2016). In the SE brain, astrocytic glutamate release leads to excessive accumulation in extracellular spaces (Fitch and Silver, 2008; Seifert et al., 2010; Sofroniew and Vinters, 2010). Increased levels of glutamate will increase neuronal activity and enhance excitotoxicity by production of ROS/RNS (Ravizza et al., 2006a; Ravizza et al., 2006b; Vezzani et al., 2000). Therefore, anti-inflammatory agents or antioxidants may be a promising strategy to reduce neuronal damage after SE (Holtkamp, 2005; Puttachary et al., 2015; Wang et al., 2015).

Liraglutide, a Glucagon-like peptide-1 (GLP-1) analogue, has been developed clinically for the treatment of type 2 diabetes mellitus (T2DM). The drug has a good safety profile with minimal adverse reaction (Vilsboll, 2009; Madsbad et al., 2004). GLP-1 receptors (GLP-1R) are widely expressed in the brain on neurones, cell bodies and dendrites.
in the brain (Hamilton and Hölscher, 2009; Merchenthaler et al., 1999; Cork et al., 2015). The GLP-1 analogue liraglutide can cross the blood-brain barrier (BBB) and activate GLP-1Rs (Hunter and Hölscher, 2012; McClean et al., 2011). Liraglutide has shown neuroprotective effects in a range of animal models of neurodegenerative disorders, such as Alzheimer’s disease (McClean et al., 2011; Hansen et al., 2015; McClean and Hölscher, 2014), Parkinson’s disease (Badawi et al., 2017; Liu et al., 2015), traumatic brain injury (Li et al., 2015; Hakon et al., 2015), motor neurone disease/ALS (Li et al., 2012), or stroke (Briyal et al., 2014; Li et al., 2016). Furthermore, liraglutide has anti-inflammatory properties in models of chronic inflammation of the brain (McClean et al., 2011; McClean and Hölscher, 2014; Parthsarathy and Hölscher, 2013; Barreto-Vianna et al., 2017). A pilot clinical trial has shown protective effects in patients with Alzheimer’s disease (Gejl et al., 2016), and another pilot study has shown improvements in cognitive performance and in MRI brain scans of people with mood disorders (Mansur et al., 2017a; Mansur et al., 2017b). In addition, the GLP-1 receptor agonist exendin-4 has shown good protective effects in a pilot study in Parkinson’s patients (Aviles-Olmos et al., 2013; Aviles-Olmos et al., 2014). A recently published phase II placebo- controlled double blind trial confirmed the initial results and showed that exendin-4 stopped disease progression (Athauda et al., 2017). This proof of concept demonstrates that the approach of activating GLP-1 receptors as a neuroprotective treatment is valid and translates into the clinic. Therefore, liraglutide or other GLP-1 analogues have the potential to alleviate the pathological processes found in SE. We therefore tested liraglutide in the pilocarpine model of SE (Curia et al., 2008) at a dose that previously showed neuroprotective effects in the CNS (McClean et al., 2011). We show for the first time that liraglutide has clear neuroprotective properties in this animal model.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats weight 200–250 g were used. All animals were provided by the Beijing Vital River Laboratory Animal Technology. And they were housed under controlled environmental conditions (22 ± 3 °C), humidity (50–55%) and 12-h light/dark cycles with free access to food and water ad libitum. The work was licenced by the ethics committee of Shanxi province. All experimental procedures were conducted in accordance with the National Institutes of Health(NIH) guideline (National Institutes of Health Publications, No. 80-23, revised 1978).

2.2. Liraglutide

Liraglutide (Peptide Purity: 95.77%) was obtained from Chinapeptides Ltd (Shanghai, China). The purity of the peptide was confirmed by reversed-phase HPLC and characterised using matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry.

2.3. Induction of SE

The experimental rats were injected with lithium chloride (127 mg/kg, ip., Sigma-Aldrich, USA) 20 h before atropine sulfate (1 mg/kg, i.p., Tianjin Jinyao, China) which reduced the peripheral cholineric effects of pilocarpine. Then, 30 min later, pilocarpine hydrochloride (30 mg/kg, i.p., MedChem Express, USA) was administered. Each animal’s behaviour was evaluated according to Racine’s scale (Racine, 1972), and only those rats that exhibited ongoing convulsive seizures (level 4–5) for 30 min without recovery between seizures would be used as SE model. Diazepam (10 mg/kg, i.p., Tianjin Jinyao, China) was used to treat the animals to terminate the convulsive seizures one hour after onset of SE. Based on the high mortality of pilocarpine-induced SE, we would add animals to guarantee the numbers of each group.

2.4. Experimental design

The 54 rats were divided into three groups: (I) saline treated control group (n = 6); (II) pilocarpine treated group (n = 24); (III) pilocarpine + liraglutide group (n = 24). The pilocarpine group and pilocarpine + liraglutide group were divided into four subgroups (n = 6) according to the different time points (12 h, 1 days, 3days, 7days) after termination of SE. Following the termination, liraglutide (25 nmol/kg, ip.) was administered immediately once – daily in the pilocarpine + liraglutide group, while the pilocarpine group rats were treated with 0.9% NaCl saline instead. The dose of liraglutide was based on the studies showing beneficial effects in AD or PD (McClean and Hölscher, 2014; Yuan et al., 2017; McClean et al., 2015).

2.5. Glucose measurements

Blood samples from the tail vein were used for the glucose test. Blood glucose levels were tested before sacrifice at different time points (12 h, 1 d, 3 d, 7 d after termination of SE) by a Sannuo blood glucose meter (Sinocare Inc. China). Repeated measurements were taken three times to reduce error and the average was taken as the blood glucose level.

2.6. Western blot

Three rats in each subgroup were sacrificed. Rats were anesthetized with 5% chloral hydrate (5 mL/kg, ip.). After cardiac perfusion with cold 0.9% NaCl, the brain was dissected on ice and hippocampi were removed. Hippocampus tissue was homogenised in ice cold RIPA buffer (Beyotime Institute of Biotechnology, China) added phenyl-methylsulfonyl fluoride (PMSF) and centrifuged 12000 rpm/min (=3200g) for 10 min at 4 °C. Then, the supernatant was taken for detection of protein concentration by BCA Protein Assay Kit (Boster Institute of Biotechnology, China). After supernatant was mixed with loading buffer and boiled for 10 min, every sample that contained 20 ug protein was separated by 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDA-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes. Next, the membranes were blocked with 5% non-fat milk in 4 °C for one night to prevent nonspecific binding. Primary antibodies incubated overnight at 4 °C with rabbit anti-rat Bet-2 (1:1000, bioworld technology, USA), rabbit anti-rat Bax (1:1000, Bioworld technology, USA), rabbit anti-rat IL-1β (1:1000, bioworld technology, USA), rabbit anti-rat TNF-α (1:1000, Abcam, UK) and rabbit anti-rat β-actin (1:3000, Bioworld, USA) diluted in PBS. After washing three times (10 min per wash) in TBST, membranes were incubated in goat anti-rabbit IgG with horseradish peroxidise conjugate (1:3000, Boster Institute of Biotechnology, China) for 2 h and then washed three times in TBST. The protein bands were detected by ECL-enhanced chemiluminescence (Boster Institute of Biotechnology, China).
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