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LARETH-25 and β-CD improve central transitivity and central pharmacological effect of the GLP-2 peptide

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ABSTRACT

Depression is a common mental disorder. More than 350 million people of all ages suffer from depression worldwide. Although a number of antidepressants are available, >20% of patients with major depressive disorder suffer from treatment-resistant depression. Therefore, development of novel therapeutics to overcome this condition is required. We reported that intracerebroventricular administration of glucagon-like peptide-2 (GLP-2) exerts antidepressant-like effects treated with or without adrenocorticotropic hormone. In the present study, we developed a nasal formulation of GLP-2 containing 5% polyoxyethylene (25) lauryl ether and 1% β-cyclodextrin that enhanced the resistance of GLP-2 to inactivation by dipeptidyl peptidase-4. Intranasal administration of this formulation (60 μg/kg) increased the delivery of GLP-2 to the brain and had antidepressant-like effects on rats. These results suggest the potential of the GLP-2 nasal formulation for use as a novel antidepressant.

1. Introduction

According to the World Health Organization, depression is a common mental disorder that affects >350 million people of all ages worldwide, and its incidence is predicted to increase in future. Although a number of antidepressants are available, >20% of patients with major depressive disorder suffer from treatment-resistant depression (Li et al., 2007). Therefore, the development of novel therapeutics for this condition is required.

In recent years, neuropeptides have attracted attention as new therapeutics for treating central nervous system (CNS) disorders, including depression. Peptides are ubiquitously present in the body and mediate a variety of physiological functions. However, there are several barriers to the uptake of peptides by the brain. For example, the blood–brain barrier (BBB) segregates the interstitial fluid of the brain from the systemic circulation, and the blood–cerebrospinal fluid (CSF) barrier (BCSFB) separates blood from the CSF that bathes the brain. These barriers prevent the diffusion of drugs, particularly polar drugs such as peptides and proteins, from the blood stream into the CNS (Charlton et al., 2007). Moreover, peptides are subjected to hydrolysis and aggregation, and these factors hinder the development of peptide therapeutics. Therefore, peptides designed to treat CNS disorders must be highly stable and deliverable directly into the brain. To address these issues, we focused first on direct drug delivery to the brain via the nasal mucosa (Lochhead and Thorne, 2012).

Studies using animals and humans show that drugs are transported directly from the nasal cavity to the CNS via the olfactory epithelium, the olfactory and trigeminal nerves, and/or

Abbreviations: β-CD, β-cyclodextrin; LAURETH-25, Polyoxyethylene (25) lauryl ether; GLP-2, Glucagon-like peptide-2; DPP-4, Dipeptidyl Peptidase-4; BBB, Blood–Brain Barrier; Amy, Amygdala; CNS, Central nervous system; CMC, Critical Micelle Concentration.

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the BBB and BCSFB. Because these routes such as nasal treatments bypass the olfactory and trigeminal nerves and require the permeability of the BBB and BCSFB membranes, few peptides meet these criteria. Therefore, we focused on surfactants and cyclodextrin to enhance the permeability of nasal formulations of peptides. The olfactory epithelium functions as a tight junction barrier that is impermeable to molecules approximately >1000 Da. In contrast, nasal delivery of macromolecular drugs such as insulin is achieved by enhancing the tight junction permeability (Seki, 2012). Therefore, this route is adaptable to every peptide because of the interface action of the surfactant. Because cyclodextrin protects peptides from degradation (Lange and Gierlach-Hladon, 2015), we reasoned that specific cyclodextrin–peptide inclusion complexes might inhibit proteolysis.

Here we focused on the 33 amino acid residue peptide glucagon-like peptide-2 (GLP-2), which is produced from proglucagon in the gut and CNS in rodents and human (Mojsov et al., 1986; Dhanvantari et al., 1996). The gene encoding the GLP-2 receptor is expressed in distinct gastrointestinal cells (Munroe et al., 1999; Yusta et al., 2000; Bjerknes and Cheng, 2001) and in specific regions of the CNS, including the dorsomedial hypothalamic nucleus (DMH), amygdala (Amy), thalamus, cerebellum, hippocampus, and cerebral cortex (Tang-Christensen et al., 2001; Lovshin et al., 2004). The central administration of GLP-2 to rodents suppresses food intake (Tang-Christensen et al., 2000) and decreases blood pressure (Sasaki-Hamada et al., 2012). Further, the intracerebroventricular (i.c.v.) administration of GLP-2 exerts antidepressant-like effects in rodents as well as imipramine-resistant depression-model animals (Iwai et al., 2009, 2013b; Sasaki-Hamada et al., 2015). Although we were highly optimistic about the possible therapeutic use of GLP-2 as a new antidepressant, i.c.v. administration is not suitable for patients. Therefore, other robust methods of delivering GLP-2 to the brain are required.

However, because the BBB commonly prevents effective treatment with most oral or intravenous drugs, with few exceptions, it represents a major obstacle to the development of therapeutic agents for CNS disorders. Therefore, as mentioned above, we focused on drug delivery to the brain through the nasal mucosa and tested nasal formulations containing a surfactant and cyclodextrin that may be suitable for efficient and noninvasive delivery of GLP-2 to the brain. The estimated elimination $t_{1/2}$ of exogenously administrated GLP-2 determined in human studies is approximately 7 min (Hartmann et al., 2000), and GLP-2 must be protected from degradation by dipeptidyl peptidase-4 (DPP-4). In our nasal formulations, we therefore attempted to improve drug stability and promote mucosal absorption by employing the nonionic surfactant polyoxyethylene (25) lauryl ether (LAURETH-25) and β-cyclodextrin (β-CD). Moreover, because β-CD increases the central distribution of a GLP-1 antagonist and improves the stability of certain peptides (Banks et al., 2004), we reasoned that β-CD might inhibit the degradation of GLP-2 through the formation of a cyclodextrin–peptide inclusion complex (Lange and Gierlach-Hladon, 2015) that might improve the peptide's distribution in the CNS (Nonaka et al., 2012).

2. Materials and methods

2.1. Materials

LARETH-25(BL-25) JP grade, (Nikko Chemicals Co., Tokyo. Japan), β-CD (C6H10O5)7 :GR(Guaranteed Reagent), (NACALAI TESQUE,INC Kyoto, Japan) DPP4 (CD26) (39–766), His-tagged, Human, Recombinant (Funakoshi Co, Tokyo, Japan) TFA(trifluoroacetic acid), Wako Special Grade, (Wako Pure Chemical Industries Osaka, Japan)

2.2. Methods

2.2.1. In vitro *studies*

2.2.1.1. Analysis of particle size. The size distribution of nanoparticles was analyzed using dynamic light-scattering spectroscopy at a fixed angle of 90° at 25 °C (ELSZ-2, Otsuka Electronics Co., Osaka, Japan).

2.2.1.2. Stability tests of GLP-2 formulations. GLP-2 peptide dissolved in phosphate buffer solution (pH 7.6) at 4 °C. And we mixed β-CD and LAURETH-25 with GLP-2 solution at 4 °C.

We evaluated the stabilities of the GLP-2 formulations GLP-2 (Peptide Inc., Osaka, Japan) as follows: GLP–2 + LAURETH-25 (CAS No. 9002-92-0, $C_{58}H_{118}O_{24}$) (Nikko Chemicals Inc., Tokyo, Japan); GLP-2 + β-CD (CAS No. 7585-39-9, $C_{42}H_{70}O_{35}$) (Nacalai Tesque Inc., Kyoto, Japan); and GLP–2 + LAURETH-25 + β-CD. Stability was determined using high-performance liquid chromatography (HPLC) (LC-20A series; Shimadzu, Kyoto, Japan) and calculated as the percentage of intact GLP-2 at 4 °C after 4 h. The amount of GLP-2 present at 0 h was defined as 100%. HPLC conditions were as follows: separation was achieved using a reversed-phase column (C18, 100 × 4.6 mm; Sigma-Aldrich, St. Louis, Mo, USA). Phase A was 0.1% trifluoroacetic acid (TFA) in water and phase B was 0.085% TFA solution in acetonitrile. Samples were eluted with a linear gradient of acetonitrile (25%–45%) delivered at 1.0 ml/min at 40 °C, and UV absorbance was detected at 214 nm.

2.2.1.3. Assays of DPP-4. Because GLP-2 is degraded by DPP-4 *in vivo* (Hartmann et al., 2000), we performed stability tests of its degradation mediated by DPP-4. To evaluate the stability of our GLP-2 formulations (GLP-2, GLP–2 + LAURETH-25, GLP-2 + β-CD, and GLP–2 + LAURETH-25 + β-CD) in the presence of DPP-4, we performed kinetic analysis using HPLC. We assessed the stability of each GLP-2 formulation by calculating the percentage of intact GLP-2 at 37 °C after 0, 1.5, and 6 h, and the amount of GLP-2 present at 0 h was defined as 100%. Whereas the stability evaluation of GLP −2 *in vivo* evaluated stability of GLP −2 at 37 °C using DPP −4 that was a main degradation enzyme of GLP −2.

We diluted the GLP-2 formulations by 200-fold and added DPP-4 (0.0214 mU). HPLC conditions were the same as those described above.

2.2.1.4. The cytotoxic evaluation of LARETH-25 and β −CD in cell line. We conducted the cytotoxic evaluation with the additive (LAURETH-25, β-CD) which we used for GLP-2 preparation. We cultured A549 of 2×10^4 cells on 96 well plates and incubation (37 °C, 5% CO_2) for 24 h. We adjusted the definitive exposure level of reagent which evaluated injury characteristics to the concentrations (5% LAURETH-25, 1% β-CD) at the nasal treatment and incubation (37 °C, 5% CO_2) for 15 min.

We measured LDH according to protocol of Cytotoxicity Detection KitPLUS (LDH) and evaluated cell cytotoxicity. We used PBS for a negative control. And we used Polyethylene Glycol Mono-*p*-isooctylphenyl Ether for a positive control in this examination.

2.2.2. In vivo *studies*

2.2.2.1. Animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science and were conducted according to the guidelines of the National Institute of Health and Japan Neuroscience Society (The number of approved protocols were "Y13019, Y14021, Y15040".). We used male Wistar rats (8–10 weeks) (Japan SLC, Shizuoka, Japan) and attempted to minimize the number of animals used and their suffering. All animals were kept in a controlled environment