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Preventive effect of rikkunshito on gastric motor function inhibited by L-dopa in rats

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ABSTRACT

We previously reported that ghrelin prevented L-dopa (LD)-induced inhibition of gastric emptying (GE) of a non-nutrient solution in rats. Parkinson's disease treatment involves the combined administration of L-dopa with the enzyme L-amino acid decarboxylase inhibitor, carbidopa (CD) to reduce peripheral formation of dopamine. We investigated the effect LD/CD given orogastrically (og) on GE of a non-nutrient or nutrient meal and whether og pretreatment with rikkunshito, a kampo medicine clinically used to treat gastroparesis, influenced LD/CD effect on GE and postprandial antral and duodenal motility in conscious rats. LD/CD (20/2 mg kg^{-1}) decreased significantly GE to 26.3 ± 6.0% compared to 61.2 ± 3.2% in og vehicle monitored 20-min after a non-nutrient meal and to 41.9 ± 5.8% compared to 72.9 ± 5.2% in og vehicle monitored 60 min after a nutrient meal. Rikkunshito (0.5 or 1.0 g kg^{-1}) reduced the LD/CD (20/2 mg kg^{-1}) inhibition of GE of non-nutrient meal (36.9 ± 7.4% and 46.6 ± 4.8% respectively vs. 12.1 ± 7.4% in og vehicle plus LD/CD) while having no effect alone (56.6 ± 8.5%). The ghrelin antagonist, [D-Lys^3]-GHRP-6 (1 mg kg^{-1}) injected intraperitoneally partially reversed rikkunshito preventive effect on LD/CD-inhibited GE. Rikkunshito (1.0 g kg^{-1}) blocked LD/CD (20/2 mg kg^{-1})-induced delayed GE of a nutrient meal and the reduction of postprandial antral motility. In 6-hydroxydopamine-induced Parkinson's disease rat model, rikkunshito (1.0 g kg^{-1}, og) also prevented LD/CD-inhibited gastric emptying of a nutrient meal and enhanced fasting plasma levels of acylated ghrelin. These data indicate that oral rikkunshito alleviates the delayed GE induced by LD/CD in naïve and PD rat model in part through ghrelin-related mechanisms.

1. Introduction

Oral administration of L-dopa (levodopa), a metabolic precursor of dopamine, is regarded as the "gold standard" for the treatment and management of Parkinson's disease (PD), a pathological condition associated with progressive degeneration of nigrostriatal dopamine pathway [37]. Unlike dopamine, L-dopa crosses the blood brain barrier via a saturable transporter and is converted to dopamine in the brain by the enzyme, L-amino acid decarboxylase (AADC) to produce its therapeutic effect [8]. However, the high pre-systemic metabolism of L-dopa to dopamine within the gut including the stomach by AADC can reduce up to 70% the initial oral dose of L-dopa that will undergo active transport and absorption by the small intestine [8,36]. Therefore, L-dopa administered orally is given in combination with a peripheral AADC inhibitor, commonly, carbidopa (α-methyldopahydrazine) at a ratio of 10/1 or 4/1 to curtail the gastrointestinal conversion of L-dopa to dopamine and consequently maximize L-dopa entry into the brain [11].

One determinant factor that also influences the L-dopa bioavailability is the gastric emptying rate. Clinical studies provided evidence of a relationship between L-dopa pharmacokinetics and gastric emptying in PD patients [15,32]. The oral administration of L-dopa/carbidopa (LD/CD) given in the fasting state or before a low protein meal inhibits gastric emptying in healthy young or elderly volunteers [40–42,57] as well as in PD patients who have already

Abbreviations: AUC, area under the curve; AADC, L-amino acid decarboxylase; DW, distilled water; GE, gastric emptying; LD/CD, L, -dopa/carbidopa; og, orogastric or orogastrically; %MI, percentage change in motility index; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease.

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developed delayed solid gastric emptying [12,19,24]. In contrast to clinical studies, there is a paucity of experimental studies on the effects of L-dopa on gastric motor function [53,60] and the influence of peripheral AADC inhibitors administered orally in conjunction with L-dopa is still unknown in rodents.

To improve the management of altered gastric emptying in PD patients treated with anti-parkinsonian drug therapy, a few gastric prokinetic agents such as the serotonin receptor 4 (5-HT_4) agonist, cisapride have been shown effective [3,38]. However, the cardiac arrhythmia side effects of cisapride lead to its withdrawal from the market and limited its clinical use [38]. Dopamine antagonists such as domperidone, a dopamine D2 receptor antagonist that does not readily cross the blood brain barrier [23], has been reported to accelerate gastric emptying of a solid meal [39,46] and to increase plasma L-dopa concentration [35] in PD patients treated with L-dopa/AACD inhibitors. However domperidone is not licensed in every country and safety issue has been recently pointed out due to potential cardiotoxic effects at high dose in elderly patients [26]. Another potential candidate is ghrelin (acylated, "active" form), a gut peptide hormone that has potent gastric prokinetic effects [9]. Our recent preclinical studies indicate that ghrelin prevented oral administration of L-dopa-induced delayed gastric emptying of a non-nutrient solution in rats [60]. Stable ghrelin agonists have been reported to improve delayed gastric emptying in various experimental and clinical conditions associated with diabetes, postoperative and morphine-induced ileus and immune challenge [4,7,33,48,56,58]. Likewise, rikkunshito, a Japanese Kampo medicine acting as a ghrelin enhancer [50], alleviates gastroparesis [1,52], dyspepsia [1,25,52,54,61], post-operative gastric ileus [27,62], and gastroesophageal reflux disease [31,34,54] in experimental or clinical studies. A recent pilot clinical study also indicates that rikkunshito can ameliorate gastroparesis in PD patients [14].

Therefore, the objectives of the present study were first to test the influence of orogastric (og) administration of L-dopa in conjunction with carbidopa on gastric emptying of non-nutrient and nutrient meals, and postprandial antro-duodenal motility in conscious rats. Second, to examine whether orally administered rikkunshito ameliorates LD/CD-induced alterations of gastric motor function and whether rikkunshito action involves ghrelin signaling using the receptor antagonist, [D-Lys^3]-GHRP-6 [2]. Lastly, we used the 6-hydroxydopamine (6-OHDA) experimental PD model [45] treated with LD/CD to assess the effects of rikkunshito on gastric emptying and plasma ghrelin levels.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Harlan, San Diego, CA, USA, weighting 280–320 g and Charles River Laboratories Japan, Yokohama, Japan, weighting 230–290 g) were housed 2–4 animals/cage under controlled illumination (12:12 h light/dark cycle) and temperature ($22 \pm 2\,^{\circ}C$) and acclimatized for at least one week before the experiments. Animals were fed standard rodent diet (Prolab RMH 2500, LabDiet, PMI Nutrition, Brentwood, MO, USA and MF, Oriental Yeast, Tokyo, Japan) and tap water ad libitum. In other studies, 6-OHDA and vehicle microinjected rats were purchased from Japan SLC (Shizuoka, Japan) 5 weeks after treatment. Eight-weeks old Sprague-Dawley male rats (Japan SLC) were microinjected into the right striatum with either vehicle (0.2% ascorbic acid/saline, $2\,\mu L \times 4$ sites) or 6-OHDA (Sigma-Aldrich, USA, $3.5\,\mu g\,\mu L^{-1}$ in 0.2% ascorbic acid/saline, $2\,\mu L \times 4$ sites) using the following coordinates from bregma: anterior–posterior (+1.3, +0.4, −0.4, −1.3 mm), mediolateral (−2.6, −3.0, −4.2, −4.5 mm) dorsoventral (−5.0 mm). After surgery, all rats were kept one per cage and 4 weeks later, the 6-OHDA rat were tested for behavioral manifestations of PD assessed by more than seven rotations/min in response to a subcutaneous injection of apomorphine ($0.5\,mg\,kg^{-1}$). One week after the apomorphine test, 6-OHDA and control rats were received at the experimental facilities. They were housed 2/cages and acclimated to similar conditions as the naïve rats for another week before the experiments, and their respective body weight was 380–440 g and 370–460 g.

Animal care and experimental procedures followed institutional ethic guidelines and conformed to the requirements of the federal authority for animal research conduct. All procedures were approved by the Animal Research Committee at Veterans Affairs Greater Los Angeles Healthcare System (animal protocol #06015-08) and Experimental Animal Ethics Committee of Tsumura & Co (animal protocol #12-028 and #12-128).

2.2. Compounds

Rikkunshito, powdered extract consisting of *Atractylodis lanceae rhizoma* (4 g, 18.6%), *Ginseng radix* (4 g, 18.6%), *Pinelliae tuber* (4 g, 18.6%), *Hoelen* (4 g, 18.6%), *Zizyphi fructus* (2 g, 9.3%), *Aurantii nobilis pericarpium* (2 g, 9.3%), *Glycyrrhizae radix* (1 g, 4.7%), *and Zingiberis rhizoma* (0.5 g, 2.3%) (Tsumura & Co., Tokyo, Japan) was suspended in water. L-dopa (L-3,4-dihydroxyphenylalanine methyl ester hydrochloride) and s-(−)-carbidopa (CD) were dissolved in vehicle composed of 10% dimethyl sulfoxide, 5% Tween-80 and 85% saline, all from Sigma-Aldrich. [D-Lys^3]-GHRP-6 (Phoenix Pharmaceuticals, CA, USA) was dissolved in sterile saline.

2.3. Gastric motor function assessment

2.3.1. Gastric emptying of a non-nutrient meal

Gastric emptying of a non-nutrient meal (1.5% methylcellulose and 0.05% phenol red viscous solution) was determined as described in our previous studies [60]. In brief, rats were fasted overnight (1 rat/cage) for 18–20 h with access to water up to the start of the experiments conducted between 9:00 AM and 1:00 PM. Animals received an orogastric gavage (og) of the viscous solution (1.5 mL) and were euthanized 20 min later by CO_2 inhalation followed by thoracotomy. The stomach was removed and homogenized in 100 mL of 0.1 N NaOH using a Polytron (Brinkman Instruments, Westbury, NY). Five milliliters of the supernatant were added to 0.5 mL 20% trichloroacetic acid, centrifuged at 3000 rpm at 4 °C for 20 min and 3 mL of the supernatant added to 4 mL of 0.5 N NaOH. The absorbance of the samples was read at 560 nm (Shimadzu 260 Spectrophotometer). Gastric emptying was calculated as percent emptying = (1 − absorbance of test sample/absorbance of standard) × 100. Phenol red recovered from stomach of rats euthanized immediately after gavage of the same volume of solution served as standard.

2.3.2. Gastric emptying of a nutrient meal

Gastric emptying of nutrient meal was performed as previously described [30]. Twenty four hours-fasted rats (2–4 rats/cage), with free access to water up to the start of the experiments conducted between 1:00 and 4:00 PM, were gavaged with 1 mL of the meal composed of standard powdered chow (32 g, MF; Oriental Yeast, Tokyo, Japan) and 40 g of glass bead (0.2-mm diameter, BZ-02; AS One, Osaka, Japan) in 80 mL of distilled water. Rats were euthanized under isoflurane anesthesia 1 h after the gavage of the meal. The stomach was removed and gastric content recovered, dried and weighed. The gastric emptying was calculated as percent emptying = (1 − dried weight of gastric content/dried weight of 1 mL test meal) × 100.