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Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction

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ABSTRACT

Circulating ghrelin concentration regulates appetite behavior, but no study thus far has focused on the role of central ghrelin in anorexia after chemotherapy. To clarify the action mechanisms of rikkunshito (RKT), a traditional Japanese medicine, on cisplatin-induced anorexia, we attempted to elucidate its effect on hypothalamic ghrelin receptor expression in cisplatin-induced anorexia. We first examined the effects of an intracerebroventricular (ICV) injection of exogenous ghrelin on food intake with or without cisplatin treatment, and the effects of cisplatin or *m*-chlorophenylpiperazine (mCPP), a 5-HT2C receptor agonist, on hypothalamic growth hormone secretagogue receptor 1a (GHS-R1a) mRNA expression. To identify the mechanism of cisplatin-induced decrease in hypothalamic GHS-R1a mRNA expression, we evaluated the effects of SB242084HCl, a 5-HT2C receptor antagonist, and RKT on hypothalamic GHS-R1a gene expression, along with the effect of coadministration of a GHS-R1a antagonist on decreased food intake. Compared to vehicle controls, an ICV-injected rat ghrelin failed to inhibit the decrease in food intake in cisplatin-treated rats. Hypothalamic GHS-R1a gene expression was significantly reduced after cisplatin or mCPP treatment, and the induced decrease was reversed by SB242084HCl or RKT, but not granisetron or ondansetron, both of which are 5-HT3 receptor antagonists. Their suppressive effect on the decrease in food intake was abolished by coadministration of the GHS-R1a antagonist. Administration of RKT or SB242084HCl reversed the decrease in food intake induced by mCPP injection. The improvement by RKT on decreased food intake after cisplatin treatment was partly mediated by hesperidin and isoliquiritigenin, components of RKT. Cisplatin-induced anorexia may worsen because of decreased hypothalamic GHS-R1a gene expression. A 5-HT2C receptor antagonist and RKT suppressed cisplatin-induced anorexia by inhibiting reduction of GHS-R1a signal transduction in the hypothalamus.

1. Introduction

Cisplatin is widely used in chemotherapy; however, undesirable gastrointestinal side effects, such as nausea, vomiting, and anorexia, markedly affect the quality of life of patients and may make continuation of chemotherapy difficult. In particular, weight loss during primary chemotherapy is a strong prognostic factor for overall survival. While some antiemetic agents have been introduced as treatment for nausea and vomiting [1,2], appetite loss is still present in 50% of cancer patients [3]. This causes high mortality and may, in fact, be the direct cause of death in some patients. However, the mechanism of the resulting appetite loss during chemotherapy has not been completely elucidated.

Ghrelin is an orexigenic hormone that has been identified as an endogenous ligand of the growth hormone secretagogue receptor (GHS-R1a) [4]. It is believed that ghrelin, which is secreted from the stomach, binds to GHS-R1a located on the ganglia of the vagus nerve and transmits a signal to the hypothalamus via vagus nerve, leading to activation of the hypothalamic appetite-stimulating system [5]. Circulating ghrelin may pass through the blood brain barrier (BBB) and bind to hypothalamic GHS-R1a [6] because the arcuate nucleus in the hypothalamus does not have a strong BBB. Alternatively, ghrelin and GHS-R1a are coexpressed in the arcuate nucleus, an area that is crucial for neuroendocrine and appetite-stimulating activity [7]. In addition, an intracerebroventricular (ICV) injection of ghrelin apparently enhances appetite in rodents [8]. A decrease in food intake and body weight loss have been noted in transgenic mice expressing an antisense agent against GHS-R1a in the hypothalamus [9], while prolonged starvation [10,11] and growth hormone [12] promote GHS-R1a gene expression in the hypothalamic arcuate nuclei.

Recent studies have demonstrated the relationship between chemotherapy-induced gastrointestinal disorders and ghrelin [13–15].

Abbreviations: 5-HT, serotonin; GHS-R1a, growth hormone secretagogue receptor; NPY, neuropeptide Y; AgRP, agouti-related protein; α-MSH, α-melanocyte stimulating hormone.

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Cisplatin-treated rats show acute decreases in the concentration of circulating ghrelin and appetite loss [13], and peripheral administration of exogenous ghrelin ameliorates anorexia [13,14] and vomiting [15] induced by cisplatin. However, the contribution of hypothalamic ghrelin signal transduction in anorexia after chemotherapy remains unclear.

Administration of cisplatin causes release of a large amount of serotonin (5-HT) from enterochromaffin cells in the gastrointestinal tract. Liu et al. [16] reported that hypothalamic 5-HT concentration in cisplatin-treated rats also increases significantly. 5-HT binds to various 5-HT receptor subtypes in the central nervous system, exerting various physiological effects. Recent findings suggest that 5-HT2C receptor is involved in the regulation of appetite [17,18]. Arcuate nucleus pro-opiomelanocortin (POMC) neurons in the hypothalamus express 5-HT2C receptor, and the activation of the receptor depolarizes POMC neurons [19]. This facilitates the release of the endogenous melanocortin 4 receptor (MC4R) agonist α-melanocyte stimulating hormone, leading to the inhibition of appetite [20,21]. However, it is unclear whether activation of the hypothalamic 5-HT2C receptor could potentially mediate anorexia after chemotherapy.

Rikkunshito (RKT) is a traditional Japanese medicine that stimulates ghrelin secretion from the stomach [13,22] and ghrelin response in the hypothalamus [23], and is widely used in Japan for the treatment of gastrointestinal motor dysfunction symptoms [24–27]. Clinically, administering RKT concomitantly with antiemetics is more effective than administering antiemetics alone against anorexia during chemotherapy in advanced breast cancer patients [28]. One possible mechanism of RKT effect is via enhancement of the circulating ghrelin concentration [13]; however, further investigation is required to clarify the orexigenic mechanisms of RKT for anorexia.

We hypothesized that a reduction in hypothalamic GHS-R1a signal transduction contributes to worsening of anorexia after cisplatin treatment. In order to clarify the mechanisms of action of RKT on anorexia in cisplatin-treated rats, we evaluated the effects of administering RKT or a 5-HT2C receptor antagonist on GHS-R1a signal transduction and food intake in cisplatin-treated rats.

2. Materials and methods

2.1. Animals

Seven-week-old male Sprague–Dawley rats (Charles River Japan, Yokohama, Japan) weighing 180–200 g were used. During the experimental period, 4 or 5 animals were maintained per cage in a room with controlled temperature and humidity with a 07:00–19:00 light cycle with free access to food and water. All experiments were performed between 09:00 and 18:00 according to the guidelines established by the Experimental Animal Ethics Committee of Saitama Medical University and Tsumura & Co.

2.2. Test substance

Cisplatin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), *m*-chlorophenylpiperazine (mCPP) as a 5-HT1B/2C receptor agonist, CGS-12066A as a 5-HT1B agonist (Sigma-Aldrich Chemical Co., St Louis, MO, USA), SB242084HCl (Sigma-Aldrich Chemical Co.) as a 5-HT2C receptor antagonist, granisetron (GlaxoSmithKline, Middlesex, UK), and ondansetron (Tyugai Ltd., Tokyo, Japan) as 5-HT3 receptor antagonists were dissolved in 0.9% sterile physiological saline (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan). Rat ghrelin (Peptide Institute Inc., Osaka, Japan) and the GHS-R1a antagonist (D-Lys^3)-GHRP-6 (Bachem, Switzerland) were also dissolved in saline. Eight crude herbs, *Atractylodis lanceae rhizoma* (4 g), *Ginseng radix* (4 g), *Pinelliae tuber* (4 g), *Hoelen* (4 g), *Zizyphi fructus* (2 g), *Aurantii nobilis pericarpium* (2 g), *Glycyrrhizae radix* (1 g), and *Zingiberis rhizoma* (0.5 g), were mixed to prepare RKT. They were then extracted with hot water and spray-dried to obtain 4 g powdered extract of RKT. RKT was then suspended in distilled water and adjusted to 500 or 1000 mg/kg body weight, the concentration at which efficacy on some anorexic animal models has been reported [13,22,23]. RKT was provided by Tsumura & Co. (Tokyo, Japan). SB242084HCl, isoliquiritigenin (Tsumura & Co.), and hesperidin (LKT Laboratories, St. Paul, MN, USA), components of RKT, were dissolved in distilled water. The highest dose of SB242084HCl (10 mg/kg, p.o.) [29,30] is similar to that which inhibited cisplatin-induced anorexia [13]. The dose of isoliquiritigenin and hesperidin used in the current experiments was comparable to that used in previous studies [13,22].

2.3. Food intake

Experiments were started at 10:00 am. Rats were individually housed 7 days before the beginning of experiments and were deprived of food for 16 h with free access to water in all tests. For food intake experiments with cisplatin-treated rats, 2 mg/kg cisplatin or vehicle was administered intraperitoneally (IP), and the effects of an ICV injection of vehicle (10 μL of saline) or rat ghrelin (2 nmol in 10 μL of saline) were simultaneously determined. Following this, cumulative 6-h food intake was measured. In another experiment, vehicle or cisplatin was injected IP, and then SB242084HCl (10 mg/kg, p.o.) [13,29,30], RKT (1000 mg/kg, p.o.) [13,22,23], hesperidin or isoliquiritigenin (5 mg/kg, p.o.) [13,22] was simultaneously administered orally by gavage. Vehicle (10 μL of saline) or (D-Lys^3)-GHRP-6 (1 nmol in 10 μL of saline) was also ICV-injected, in addition to the coadministration of test drugs with cisplatin, to clarify the role of the 5-HT2C receptor on cisplatin-induced anorexia. Test drugs were also administered to saline-treated rats (instead of (D-Lys^3)-GHRP-6 and cisplatin). In addition, to clarify the antagonistic action of RKT on 5-HT2C receptor, we examined the effect of RKT on mCPP-induced anorexia. IP administration of mCPP (9 mg/kg) was performed and RKT at a dosage of 1000 mg/kg or SB242084HCl at a dosage of 10 mg/kg was simultaneously administered orally. The cumulative 1-h food intake was measured in all animals. The food intake was calculated as the difference between the food weights before and after the feeding period.

2.4. GHS-R1a and preproghrelin mRNA determination

Sixteen-hour-fasted rats were divided into groups according to uniform average body weight. Vehicle or cisplatin was IP injected to clarify the effect of cisplatin on GHS-R1a and preproghrelin mRNA expression in the hypothalamus and stomach, and the animals were sacrificed by decapitation at 2 or 6 h after cisplatin treatment. The stomach and hypothalamus were isolated and excised at 4 °C to extract mRNA. To clarify hypothalamic mRNA expression in GHS-R1a during the fasting condition, hypothalamus and stomach were isolated from 48-h-fasted and freely fed rats.

We next evaluated the role of the 5-HT2C receptor on hypothalamic GHS-R1a mRNA expression after cisplatin treatment. mCPP (3, 5, or 9 mg/kg, i.p.) [17,18,29] or CGS-12066A (5 mg/kg, i.p.) [31] was administered to 24-h-fasted rats. Each hypothalamus was harvested to detect GHS-R1a mRNA 1 h after the injection because a preliminary test indicated that the decrease in food intake after IP injection of mCPP or CGS-12066A at the same dose peaked at 1 h.

Next, we investigated the roles of the 5-HT2C receptor on the decrease in hypothalamic GHS-R1a mRNA expression in cisplatin-treated rats. Vehicle (0.2 mL of saline) or cisplatin (2 mg/kg) was injected (IP) with or without coadministration of SB242084HCl (5 or 10 mg/kg, p.o.), RKT (500 mg/kg or 1000 mg/kg, p.o.), granisetron (0.5 mg/kg, subcutaneous injection [s.c.], or ondansetron (1.0 mg/kg, s.c.). Doses of 5-HT3 receptor antagonists improved the delay in gastric emptying in cisplatin-treated rats in independent tests (non-treated rats, 85.6 ± 1.7%; cisplatin-treated rats, 59.4 ± 6.2%, $p = 0.002$, vs. non-treated rats + 0.5 mg/kg granisetron, 83.5 ± 4.6%, $p = 0.014$, vs. cisplatin + 1.0 mg/kg ondansetron, 84.0 ± 5.7%, $p = 0.014$, vs. cisplatin alone by Dunnett's analysis). Rats were sacrificed