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The gene expression of the hypothalamic feeding-regulating peptides in cisplatin-induced anorexic rats

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ABSTRACT

Cisplatin has been widely used; however, various disadvantageous side effects afflict patients. Rikkunshito (RKT), a traditional Japanese herbal medicine, has been widely prescribed in Japan to improve anorexia; but the mechanisms are unknown. Here we studied whether RKT could improve anorexia induced by cisplatin and changes in feeding-regulating peptides in the hypothalamus in rats. Adult male rats were divided into 4 groups: water + saline (WS), water + cisplatin (WC), RKT + saline (RS), and RKT + cisplatin (RC) groups. Water or RKT (1 g/kg) was intragastrically administered for 4 days, from day −1 to day 2, and saline or cisplatin (6 mg/kg) was intraperitoneally (i.p.) administered at day 0. After i.p. administration, cumulative food intake, water intake, urine volume and body weight were measured. The rats were then decapitated, followed by removal of the brain, and feeding-regulating peptides in the hypothalamus were measured by *in situ* hybridization histochemistry. In the three-day measurements, there were no significant changes in cumulative water intake and urine volume. The body weight and cumulative food intake in WC significantly decreased compared to WS, whereas these were not observed in RC. *Pro-opiomelanocortin* (*POMC*) and *cocaine and amphetamine-regulated transcript* (*CART*) in the arcuate nucleus (ARC) in WC significantly increased, and *neuropeptide Y* (*NPY*) in the ARC decreased compared to WS, whereas those in RS and RC were comparable to WS. These results suggest that RKT may have therapeutic potential for anorexia induced by cisplatin.

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1. Introduction

Cisplatin is widely used for a variety of malignant tumors. It demonstrates anti-tumor effects by inhibiting the replication of DNA [7], but it has various side effects, such as loss of appetite, nausea and vomiting. It has been suggested that serotonin receptors are involved in the occurrence of nausea and vomiting from the use of cisplatin [17]. 5-HT3 receptor antagonist, steroids and metoclopramide have been used for the treatment of nausea and vomiting caused by cisplatin. However, in Japan, Rikkunshito (RKT) has also been used empirically for patients who suffer from anorexia caused by cisplatin.

RKT, a traditional Japanese herbal medicine, or "kampo", is widely prescribed in Japan for the treatment of the various disorders, such as upper gastrointestinal symptoms in patients with functional dyspepsia, gastroesophageal reflux disease, dyspeptic symptoms in postgastrointestinal surgery patients, and chemotherapy-induced dyspepsia in cancer patients [6,15,16,20,23]. The largest component of RKT is "Hesperidin" [22], which is a polyphenol that is contained in the peels of some kinds of oranges. It has been reported in *in vitro* experiments that RKT could act as an antioxidant [4].

Recent studies have revealed that RKT administration stimulates peripheral ghrelin secretion [3] or selective serotonin reuptake inhibitor [3,19] in rats with anorexia induced by cisplatin. Yakabi et al. demonstrated that cisplatin-induced anorexia is due to reduced ghrelin secretion in the hypothalamus of rats [26]. However, there are few studies about the mechanism of RKT for cisplatin-induced anorexia, and details of its actions have not been elucidated.

Here we studied the effects of RKT on cisplatin-induced anorexia in rats. We also assessed the impact of RKT and cisplatin on the feeding-regulating peptides in the hypothalamus.

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2. Materials and methods

2.1. Animals

Adult male Wistar rats (260–290 g body weight) were individually housed and maintained in temperature controlled (23–25 °C) conditions under a 12.12 h light/dark cycle (lights on 07.00 h). All experiments were performed in strict accordance with guidelines on the use and care of laboratory animals issued by the Physiological Society of Japan, and were approved by the Ethics Committee of Animal Care and Experimentation of University of Occupational and Environmental Health.

2.2. Test substance

RKT (Tsumura & Co., Tokyo, Japan) includes eight crude herbs (*Atractylodis lanceae rhizome*, *Ginseng radix*, *Pinelliae tuber*, *Hoelen*, *Zizyphi fructus*, *Aurantii nobilis pericarpium*, *Glycyrrhizae radix* and *Zingiberis rhizoma*). These were mixed and extracted with hot water and then spray-dried to make a RKT powdered extract. The RKT was dissolved in tap water (0.1 g/mL) for intragastrical administration. Cisplatin (Sigma–Aldrich Japan Co. LLC., Tokyo, Japan) was dissolved in 0.9% sterile physiological saline (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) (0.6 mg/1 mL).

2.3. Experimental procedure

All the rats had access to food and water *ad libitum* throughout the experiments. The rats were divided into four groups: water + saline (WS, $n = 7$), water + cisplatin (WC, $n = 7$), RKT + saline (RS, $n = 8$), and RKT + cisplatin (RC, $n = 8$). Water (1 mL/100 g bode weight) or RKT (0.1 g/1 mL/100 g body weight) were administered directly to the stomach using sondes per os. These were administered from day −1 to day 2 (16.00–18.00 h). Saline (1 mL/100 g body weight) or cisplatin (0.6 mg/1 mL/100 g body weight) were administered intraperitoneally only one time 1 h after administration of water or RKT on day 0. Body weights were measured from day −1 to day 3 every 24 h. Food and water intake were measured from day 0 to day 3 every 24 h.

After the treatment, at day 3, the rats were decapitated immediately without being anesthetized, followed by removal of the brain promptly onto dry ice, then storing at −80 °C. Trunk blood samples were taken during decapitation, and were collected into chilled reaction tubes (Greiner Bio-One) containing an aprotinin/EDTA mixture. Blood samples were centrifuged for 10 min at 4 °C, 3000 rpm. After the blood was centrifuged, a 15 μL sample of plasma was taken for measuring plasma osmolality (P-Osm) using a ONE-TEN osmometer (FISKE, Norwood, MA, USA), 10 μL for measuring plasma glucose using a Medisafe Reader GR-101 (TERUMO, Tokyo, Japan), 500 μL for measuring plasma corticotrophin (SRL, Tokyo, Japan), and 500 μL for measuring plasma active and desasyl ghrelin.

2.4. In situ hybridization histochemistry

The removed brains were cut into 12 μm thickness, and thaw mounted on gelatin/chrome alum-coated slides. The locations of the hypothalamic areas, including the paraventricular nucleus (PVN), arcuate nucleus (ARC) and lateral hypothalamic area (LHA), were determined according to coordinates of the rat brain atlas. ^{35}S 3′-end-labeled deoxyoligonucleotide complementary to transcripts encoding *oxytocin*, *corticotrophin releasing hormone* (*CRH*), *pro-opiomelanocortin* (*POMC*), *cocaine and amphetamine-regulated transcript* (*CART*), *neuropeptide Y* (*NPY*), *agouti-related protein* (*AgRP*), *melanin-concentrating hormone* (*MCH*) and *orexin* were used (*oxytocin* probe sequence, 5′-CTC GGA GAA GGC AGA CTC AGG GTC GCA GGC-3′; *CRH* probe sequence, 5′-CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC-3′; *POMC* probe sequence, 5′-TGG CTG CTC TCC AGG CAC CAG CTC CAC ACA TCT ATG GAG G-3′; *CART* probe sequence, 5′-TCC TTC TCG TGG GAC GCA TCA TCC ACG GCA GAG TAG ATG TCC AGG-3′; *NPY* probe sequence, 5′-CAA ATG GAT GAT TGG TCA TTT CAA CAT AGA GTT GGG GGC TTG CT-3′; *AgRP* probe sequence, 5′-CGA CGC GGA GAA CGA GAC TCG CGG TTC TGT GGA TCT AGC ACC TCT GCC-3′; *MCH* probe sequence, 5′-CCA ACA GGG TCG GTA GAC TCG TCC CAG CAT-3′; and *orexin* probe sequence, 5′-TCC TCA TAG TCT GGA GGC AGG TGG AAG GGT TCC CCA CTG CTA GTG-3′).

The probe was 3′-end-labeled using terminal deoxynucleotidyl transferase and [^{35}S] dATP. The *in situ* hybridization protocol has been previously described in detail [24]. Briefly, sections were fixed in 4% (w/v) formaldehyde for 5 min and incubated in saline containing 0.25% (v/v) acetic anhydride and 0.1 M triethanolamine for 10 min and then dehydrated, delipidated in chloroform, and partially rehydrated. Hybridization was carried out overnight at 37 °C in 45 μL of hybridization buffer under a Nescofilm (Bando Kagaku, Osaka, Japan) cover slip. A total count of 1×10^5 c.p.m. for oxytocin transcripts and 1×10^6 c.p.m. for CRH, POMC, CART, NPY, AgRP, MCH and orexin transcripts and per slide were used. After hybridization, sections were washed 4 times with SSC (150 mM NaCl and 15 mM sodium citrate) for 1 h at 55 °C and for an additional hour with two changes of SSC at room temperature. Hybridized sections containing hypothalamus were exposed for autoradiography (Hyperfilm, Amersham, Bucks, UK) for 6 h for oxytocin probe, 5 days for MCH and orexin probe, and 1 week for CRH, POMC, CART, NPY and AgRP probe. The resulting images were analyzed by computerized densitometry using a MCID imaging analyzer (Imaging Research Inc., Ontario, Canada). The mean optical densities (OD) of the autoradiographs were measured by comparison with simultaneously exposed ^{14}C-labeled microscale samples (Amersham, Bucks, UK) and represented in arbitrary units setting the mean OD obtained from control rats.

2.5. Statistical analysis

The mean ± SEM was calculated from the results of the body weight change, cumulative water and food intake, cumulative urine volume, and *in situ* hybridization histochemistry studies. In the results of *in situ* hybridization, the expression levels of the genes were expressed as a percentage of WS. All data were analyzed by one-way ANOVA followed by a Bonferroni-type adjustment for multiple comparisons (Origin Pro version 8.5J, Lightstone, Tokyo, Japan). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Changes in body weight

The body weight of each group was measured from day 0 to day 3 (Fig. 1A). The body weight gradually increased during the experiments, except for WC (Fig. 1A). The body weight in WC at day 3 was significantly difference in comparison with all the other groups. Data are also presented as percentage from day 0 (Fig. 1B). The results of body weight in WC presented as percentage was also significantly different in comparison with all the other groups.

3.2. Water intake, urine volume, food intake

Cumulative water intake and cumulative urine volume were measured from day −1 to day 3 (Fig. 2A and B). There were no significant differences in cumulative water intake (Fig. 2A) or cumulative urine volume (Fig. 2B) among all the experimental groups.

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