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Enhancement of ghrelin-signaling system by Rikkunshi-To attenuates teriparatide-induced pica in rats

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ABSTRACT

Teriparatide is clinically used for the treatment of osteoporosis; however, nausea is often observed in patients. Its insufficient control affects the ability to continue teriparatide therapy. Rikkunshi-To (RKT), a traditional Japanese herbal medicine, improves the gastrointestinal function via activation of the ghrelin-signaling system. We investigated the therapeutic effects of RKT on teriparatide-induced nausea in rats and the involvement of ghrelin in these effects. We previously reported that ovariectomized rats showed pica (kaolin ingestion), a behavior that can be used to assess nausea in rats, after the subcutaneous administration of teriparatide; thus, the behavior was used as an index of nausea. Ovariectomized rats were fed diets with or without RKT (1%) for 2 weeks, and then they received the subcutaneous injection of teriparatide (400 μg/kg). Teriparatide significantly increased the incidence of pica, while suppressing intestinal motility and plasma ghrelin levels in rats fed normal diets; however, rats fed diets with RKT showed improvements in all of the teriparatide-induced adverse reactions. These therapeutic effects were antagonized by a ghrelin receptor antagonist ([D-Lys^3]-GHRP-6; 200 nmol/rat). These findings suggest that the enhancement of ghrelin-signaling is involved in RKT's therapeutic effect, and that RKT is a potentially useful treatment for teriparatide-induced nausea.

1. Introduction

Parathyroid hormone (PTH), secreted from the parathyroid gland, increases serum calcium levels through the release of calcium from bones and enhancement of calcium reabsorption in the kidneys.[1] Some studies reported that the intermittent administration of PTH and its active fragment, human PTH (1–34) (teriparatide), can potentially increase the bone mineral density (BMD) and improve the skeletal microarchitecture. Thus, teriparatide has been approved as an anabolic medication for reducing the incidence of osteoporotic fractures.[2,3]

In clinical practice, one of the most common side effects of teriparatide therapy is nausea.[4] Clinical studies reported that nearly 20% of patients treated with teriparatide suffer from nausea.[2,5] Although nausea is not a life-threatening symptom, it affects the teriparatide therapy adherence rate to the extent that some patients refuse further treatment. Hypercalcemia-induced fluid and electrolyte imbalances often lead to complaints of nausea.[6] Therefore, the transient increase in calcium levels induced by teriparatide and stimulation of dopamine D_2 receptors present in the chemoreceptor trigger zone (CTZ) may be factors involved in the inhibition of gastrointestinal (GI) motility that leads to nausea.

We previously reported that pica behavior in rodents, characterized by the consumption of non-nutritive materials such as kaolin (hydrated aluminum silicate), is a behavioral index of a nausea-like response.[7–9] We also reported that rats subjected to bilateral ovariectomy (OVX), which are often used to evaluate osteoporosis drugs, showed marked pica behavior after the

Abbreviations: ANOVA, analysis of variance; BMD, bone mineral density; CMC, carboxymethylcellulose; CTZ, chemoreceptor trigger zone; GI, gastrointestinal; HCl, hydrochloric acid; HPCL, high-performance liquid chromatography; OVX, ovariectomy; PTH, parathyroid hormone (parathormone); RKT, Rikkunshi-To; RSA, rat serum albumin; SEM, standard error of the mean.

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subcutaneous administration of teriparatide compared with non-operated or sham-operated rats and that a D_2 receptor antagonist, prochlorperazine, effectively inhibited teriparatide-induced pica behavior[10]; thus, D_2 receptor antagonists are potential treatments for teriparatide-induced nausea in patients. However, another study reported that these drugs can cause extrapyramidal side effects such as hypotension, sedation, and dystonia and that they may lead to an increased risk of hip fractures in elderly patients due to falls.[11]

It is known that D_2 receptor antagonists enhance GI motility by stimulating acetylcholine release. Mok et al.[12] reported that PTH induces a reduction of GI motility and delayed gastric emptying by the relaxation of GI smooth muscle; thus, we hypothesized that improvement of GI motility by prokinetic agents other than D_2 receptor antagonists is useful for the treatment of teriparatide-induced nausea in patients. Rikkunshi-To (RKT), a traditional Japanese herbal medicine, acts as a prokinetic agent to improve the GI function via the potentiation of ghrelin secretion and its signaling pathway.[13,14] It has been widely prescribed for patients with various GI symptoms, such as anorexia, nausea, and vomiting.[15] In this study, we investigated the therapeutic effects of RKT on teriparatide-induced pica behavior in rats, and elucidated the mechanisms underlying these effects.

2. Materials and methods

2.1. Animals

Experiments were approved by the Animal Care Committee of the School of Allied Health Sciences, Faculty of Medicine, Osaka University (24-07-06), and were conducted in accordance with the Animal Experiment Guidelines of Osaka University. According to our previous report,[10] female Wistar/ST rats (300−330 g) subjected to bilateral OVX were used in this study. We requested the Biotechnical Center of Japan SLC (Shizuoka) to perform all OVX. Briefly, naïve female rats (8 weeks old) were anesthetized with 2% isoflurane (Pfizer Japan Inc., Tokyo). The dorsal surgical area was bilaterally shaved and disinfected. A small skin and muscle incision (approximately 2 cm) was made to access the peritoneal cavity, and bilateral uterine ligation was performed. The ovaries were removed via this incision. Then, the muscles and skin were closed using silk sutures. After completing the suturing, rats were administered a spray containing predonisolone and fradiomycin sulfate (Aerozolin®, Takeda Pharmaceutical, Osaka) and given 9 weeks to recover.

2.2. Effects of RKT on teriparatide-induced pica behavior in rats

To accurately measure the intakes of kaolin and food of OVX rats, we housed them in an automatic kaolin and food intake-monitoring system (FDM700SW; Melquest, Toyama).[7] This system consisted of an acrylic home cage (26 × 20 × 23), two containers (7 × 4 × 10 cm), and a controller equipped with two load cells (weight sensor). Chow pellets and hand-made kaolin pellets were provided in their respective containers. Powdered standard diet (CE-2; CLEA Japan, Inc., Tokyo) was mixed with or without 0.5, 1, or 2% (w/w) RKT (further details of RKT in chapter 2.8) in distilled water to form pellets similar in size to commercially available chow pellets. These pellets (Lots: O-5259, O-6101, O-6129, O-6346, and O-7255) were then completely dried at 80 °C for 3 h and at room temperature for 1 h. The dose of RKT was determined based on the method of Tominaga et al.[16] and the human-equivalent dose[17] with a slight modification. Kaolin pellets were prepared according to our previous method with one slight modification in the concentration of gum Arabic.[10] Pharmaceutical-grade kaolin was mixed with 5% w/w gum arabic in distilled water to form pellets similar in size to diet pellets. These pellets were then completely dried at a constant temperature (25 ± 1 °C). Both chow and kaolin intakes were recorded daily to the nearest 0.01 g using a laptop PC. Throughout the experimental period, the rats were given free access to tap water, kaolin pellets, and kaolin pellets and chow pellets with or without RKT. All rats were habituated in a room with a regular light/dark cycle (lights on from 0500 h to 1700 h) at a constant temperature (25 ± 1 °C) and humidity (50 ± 5%), and they were allowed to acclimate to the experimental environment for 2 weeks before experiments.

On the day of the experiment, they received teriparatide (400 μg/kg, s.c.) and their consumption of kaolin and food was measured for 24 h. There were eight to ten rats in each of the experimental groups. After the completion of the experiments, the rats were sacrificed with an overdose of sodium pentobarbital (150 mg/kg, i.p.). In this experiment, teriparatide was dissolved in 0.1% rat serum albumin (RSA) saline solution to prevent adsorption onto the tube surface.

2.3. Effect of RKT on teriparatide-induced intestinal motility delay in rats

The delay of intestinal motility in rats was determined based on the method of Mittelstadt et al.[18] with a slight modification. Briefly, the rats were adapted under the same experimental conditions of pica behavior. The diet (with or without 1% RKT) and kaolin pellets were provided in their respective containers for 2 weeks. The diet and kaolin pellets were removed from the containers the day before the experiment. On the day of the experiment, the rats received teriparatide (400 μg/kg, s.c.), and an intragastric injection of 100 g/L of activated charcoal suspension of 0.5% CMC solution (100 mg/kg) using a cannula. The control animals were treated with 0.1% RSA saline solution instead of teriparatide. Thirty minutes after teriparatide administration, the rats were sacrificed with an overdose of sodium pentobarbital (150 mg/kg, i.p.). Their GI tract was removed, and the distance moved by activated charcoal (beginning from the pylorus) as a percentage of the total length of the small intestine was calculated. There were nine rats in each of the experimental groups.

2.4. Effect of ghrelin receptor antagonist on RKT-induced improvement of pica behavior in rats

The rats were housed in an automatic kaolin and food intake-monitoring system and fed a diet of RKT (1%) and kaolin pellets (provided in their respective containers) for 2 weeks. Rats received a ghrelin receptor antagonist ([D-Lys^3]-GHRP-6, 100 or 200 nmol/rat/day, i.p.) twice daily for 6 days before and 1 day after receiving teriparatide (400 μg/kg, s.c.). Their daily consumption of kaolin and food was measured. Like teriparatide solution, [D-Lys^3]-GHRP-6 was also dissolved in 0.1% RSA saline solution to prevent adsorption onto the tube surface. Thus, the control animals received a twice-daily intraperitoneal injection of 0.1% RSA saline (1 mL/kg body weight, i.p.) as a vehicle. There were eight to nine rats in each of the experimental groups. After the completion of all experiments, the rats were euthanized with an overdose of sodium pentobarbital (150 mg/kg, i.p.).

2.5. Effect of ghrelin receptor antagonist on RKT-induced improvement of intestinal motility in rats

The experimental method was almost identical to that described in chapter 2.3., except RKT was provided for 2 weeks and ghrelin receptor antagonist (200 nmol/rat, i.p.) was injected twice daily for 6 days before and simultaneously with teriparatide (400 μg/kg, s.c.). Control animals received the intraperitoneal injection of 0.1% RSA

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