Influence of food on the gastric motor effect of the Kampo medicine rikkunshito in rat

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Abstract

Background: Rikkunshito, one of the Kampo medicines, is widely prescribed as a remedy for various upper gastrointestinal syndromes. The effect of rikkunshito is related to endogenous ghrelin and its active ingredient atractylodin enhances ghrelin receptor signaling. Kampo medicines are traditionally administered before or between meals; however, no definitive benefit of the timing of administration has been proven yet. To clarify the influence of food on the pharmacological action of rikkunshito, we investigated the gastric motor activity and pharmacokinetic profiles of atractylodin after the administration of rikkunshito in fasted and fed rats.

Methods: Phase III-like contractions in the gastric antrum after an injection of ghrelin were measured using a strain gauge force transducer. Rikkunshito was administered to rats during fasting or after a nutrient test meal. Ghrelin was injected 30 minutes later and gastric motility was evaluated. Furthermore, after rikkunshito administration, the pharmacokinetic profiles of atractylodin in the plasma and brain of fasted and free-fed rats were assessed.

Key Results: Rikkunshito administration potentiated ghrelin-induced phase III-like contractions under fasting conditions. This effect was attenuated in animals fed a test meal. Atractylodin was detected pharmacokinetically in the plasma and brain after rikkunshito administration in rats, and free-fed rats exhibited a decreased maximum concentration of plasma atractylodin and a delayed time to reach the maximum concentration.

Conclusions & Inferences: We show that the pharmacological action of rikkunshito is influenced by food in rats. The efficacy of rikkunshito may be associated with decreased absorption of its active ingredient atractylodin when food is in the stomach.

KEYWORDS
atractylodin, gastric motility, ghrelin, pharmacokinetics, rikkunshito

1 INTRODUCTION

Traditional Japanese medicine (Kampo medicine) has been approved by the Japanese Ministry of Health, Labour and Welfare. It has been proposed as a therapeutic approach for several disorders for which western therapies, in particular, are currently lacking. Recently, the scientific evidence for the use of Kampo medicine in clinical practice has been accumulating continuously. Especially rikkunshito is one of the Kampo medicines supported by much evidence. Several clinical studies of rikkunshito have demonstrated its effectiveness in...
anorexia, gastric dysmotility, and gastrointestinal (GI) symptoms such as postprandial fullness and epigastric pain\(^6\). Thus, rikkunshito is widely prescribed as a remedy for various upper GI syndromes.

In clinical studies\(^7,8\) and animal model experiments\(^9,12\), the effects of endogenous ghrelin have been reported as the mechanism for these actions of rikkunshito. Ghrelin is a 28-amino acid peptide identified as a natural ligand for the growth hormone secretagogue-receptor (GHS-R)\(^{13}\). It is mainly secreted from X/A-like cells in the stomach in coordination with circadian rhythms in feeding behavior, and to some extent, secreted in the brain, and plays a role in eliciting feeding and augmenting GI motility via the gut—brain axis. Rikkunshito synergistically promotes endogenous ghrelin activity by regulating ghrelin secretion\(^7\), ghrelin receptor sensitization\(^{15}\), and ghrelin degradation\(^{11}\). The active ingredients of rikkunshito responsible for these effects have been identified\(^{14}\).

A recent clinical pharmacokinetic study has demonstrated that oral administration of rikkunshito in healthy volunteers increases the plasma concentration of several active ingredients in a dose-dependent manner; atractylodin, in particular, is a major ingredient detected in the plasma\(^{15}\). In vitro studies have revealed that atractylodin increases the binding activity of ghrelin to GHS-R and sustains the increase in ghrelin-induced intracellular calcium ions or cyclic adenosine monophosphate in GHS-R-expressing cells, suggesting an enhancing activity of ghrelin receptor signaling\(^{10,16}\). Thus, atractylodin might modify appetite or GI motility.

Although almost all Kampo medicines including rikkunshito are traditionally administered before or between meals in patients, there is no definitive proof yet for a benefit to the correct timing of administration. Thus, to clarify the influence of food on the pharmacological action of rikkunshito, we focused on the ghrelin receptor-stimulating action of atractylodin and investigated its effect on gastric motor activity and on the pharmacokinetic profile of rikkunshito after oral administration under fasted and fed conditions.

2 MATERIALS AND METHODS

2.1 Animals

Male Wistar rats weighing 200-250 g were purchased from Clea Japan (Tokyo, Japan). Seven-week-old male Sprague-Dawley (SD) rats were purchased from Charles River Laboratories Japan (Tokyo, Japan). They were housed in a regulated environment, with controlled conditions of ambient temperature (23±3°C), humidity (50±20%), and lighting (12-hour light:dark cycle). Animals were given free access to water and standard laboratory chow. All experimental procedures were performed according to the “Guidelines for the Care and Use of Laboratory Animals” and approved by the Laboratory Animal Committee of Tsumura & Co. (Tokyo, Japan) (Approval number: 12-084, 12-118, 13-058, 16-039).

2.2 Reagent preparation

Ghrelin (acyl ghrelin, Peptide Institute, Osaka, Japan) was dissolved in saline. The Kampo medicine rikkunshito (Tsumura, Tokyo, Japan) is a dried and powdered herbal extract composed of the following eight constituents: Atractylodes lancea rhizome (Atractylodis lanceae rhizoma) 4.0 g, Ginseng (Ginseng radix) 4.0 g, Pinellia Tuber (Pinelliae tuber) 4.0 g, Poria Sclerotium (Poria) 4.0 g, Jujube (Zizyphi fructus) 2.0 g, Citrus Unshiu Peel (Aurantii nobilis pericarpium) 2.0 g, Glycyrrhiza (Glycyrrhizae radix) 1.0 g, and Ginger (Zingiberis rhizoma) 0.5 g. This powder was suspended in distilled water at doses of 1000 mg kg\(^{-1}\).

Atractylodin (Wako, Osaka, Japan), an active ingredient of rikkunshito, was dissolved in a solution of 0.1% ethanol and 1% Tween-80.

2.3 Animal preparation

We used male Wistar rat for motility index examination as described previously\(^{17}\). Overnight fasted rats were anesthetized with sodium pentobarbital (50 mg kg\(^{-1}\), intraperitoneally, Kyoritsu Seiyaku Corporation, Tokyo, Japan). After laparotomy, a strain gauge force transducer (F-0818S, Star Medical, Inc., Tokyo, Japan) was placed on the serosal surface of the antrum in the stomach to measure the contractions of the circular muscles. The wire of the transducer was led subcutaneously over the back to emerge at the back of the neck. A vessel catheter was inserted into the right jugular vein and emerged from the back of the neck. The catheter was filled with heparinized saline (100 units mL\(^{-1}\)) to avoid blood coagulation. The wire and catheter were led through a protective coil. Measurements were performed under free-moving conditions in individual cages after a postoperative period of 5-7 days for recovery.

2.4 Measurement of gastric motility

The connector of the strain gauge force transducer placed in the fasted rats was connected to an FS-04M preamplifier (Star Medical, Inc.) via an FB-01 bridge box (Star Medical, Inc.) to allow measurement of movement of the antrum. Data were recorded using an MP150 (BIOPAC Systems, CA, USA). The system was calibrated before each experiment using a calibrator (Star Medical, Inc.), and contractions were expressed in grams.

Key Points

- Rikkunshito is used for various upper gastrointestinal syndromes and known to promote endogenous ghrelin activity. This study aims to investigate the influence of food and timing of administration on the pharmacological action of rikkunshito.
- Rikkunshito administration potentiated ghrelin-induced gastric motor activity in fasted rats. Plasma and brain levels of atractylodin, an active ingredient of rikkunshito, increased more and more rapidly in fasted than in free-fed rats.
- The efficacy of rikkunshito may be associated with decreased absorption of atractylodin when food is in the stomach.
2.5 | Experimental protocols

The experiment was started when fasted-state gastric contraction stabilized in food-deprived rats at more than 2 hours after the initial measurement. However, spontaneous phase III-like contractions still occurred irregularly in fasted rats. Ghrelin (3 nmol) was therefore injected into the rat’s right jugular vein through a vessel catheter to induce phase III-like contractions. Subsequently, the area under the wave (motility index, MI) was measured in the antrum during 30 minutes. After 1 hour, when the stabilized fasted-state gastric contraction was restored, rikkunshito (1000 mg kg⁻¹, n=7), atractylodin (1 mg kg⁻¹, n=7), distilled water (n=7), or vehicle (n=7) was orally administered to rats, followed by a second ghrelin (3 nmol) injection 30 minutes later. MI in the antrum was measured for a 30-minute period and recorded as a percentage relative to MI (%MI) in response to the first ghrelin injection.

To assess the efficacy of rikkunshito administration in the postprandial period, fasted rats were gavaged with a nutrient test meal (1.44 kcal, 1 mL rat⁻¹), consisting of a suspension of 8 g of standard laboratory powdered chow (MF; Oriental Yeast, Tokyo, Japan) in 20 mL of distilled water, 30 minutes before the administration of rikkunshito (1000 mg kg⁻¹, n=7) or distilled water (n=7).

2.6 | Time-course plasma sampling and pharmacokinetic analysis

For the pharmacokinetic study, we used male SD rat in an international genetic standardized colony. A vessel catheter was inserted into the right jugular vein in rats under isoflurane anesthesia. After recovering for 1 week after the operation, 16-hour fasted rats (n=7) and free-fed rats (n=6) were orally administered 1000 mg kg⁻¹ of rikkunshito. Blood samples (0.5 mL) were collected through the catheter from the same animal 0, 0.25, 0.5, 1, 2, 4, and 8 hours after treatment with rikkunshito. After the blood samples were heparinized and centrifuged (3000 rpm, 15 minutes, 4°C), plasma samples were collected and stored at −80°C until use.

The level of atractylodin in plasma samples was determined according to a slight modification of the previously reported method. The plasma samples (250 μL) were mixed with 12.5 μL of methanol. Subsequently, diethyl ether (3 mL) was added to the solutions, followed by shaking and centrifugation (3000 rpm, 5 minutes, 4°C). The supernatant was dried at 25°C under a stream of nitrogen gas. Subsequently, acetonitrile (200 μL) was added to the dried samples, followed by mixing and sonication. The solutions were filtrated (0.22 μm; Millipore Corporation, Billerica, MA, USA) and centrifuged (10,000 rpm, 2 minutes, 4°C). The sample (1 μL) was injected into a gas chromatography—mass spectrometry (GC–MS) instrument, which consisted of an HP6890 series gas chromatograph connected to an HP5973N mass selective detector (Agilent Technologies, Santa Clara, CA, USA). The GC column was an HP-1 (30 m×0.25 mm id, 0.25 μm; Agilent Technologies). The temperature was set at 100°C for 5 min, raised to 250°C at 15°C per min, and maintained there for 5 minutes. The carrier gas was helium with a capillary splitless injector. The temperature of the injection port was 300°C. Atractylodin was quantified by selected ion monitoring transitions at 182 to 152 m z⁻¹.

Plasma pharmacokinetic data were analyzed by non-compartmental modeling using Phoenix WinNonlin (version 6.4, Certara L.P., St. Louis, MO, USA) to determine various pharmacokinetic constants including the maximum concentration (C,max), time to maximum concentration (t,max), and area under the plasma concentration—time curve from zero to last observation time (AUCₙₗₒₙₜ). The C,max and AUCₙₗₒₜ of atractylodin in each group are presented as the geometric mean (95% confidence interval [CI]). The tₚₘ₃ₐ data are presented as medians with a range from minimum to maximum.

2.7 | Brain sampling and pharmacokinetics analysis

Sixteen-h fasted rats (n=5) and free-fed rats (n=5) were orally administered 1000 mg kg⁻¹ of rikkunshito and decapitated 0, 0.083, 0.25, 0.5, 1, 2, 3, and 4 hours later. The brain (the cerebrum except for the cerebellum, medulla oblongata, and olfactory bulb) was collected and immediately frozen and stored at −20°C until use. The brain was homogenized after adding two volumes (w/w) of distilled water.

To quantify atractylodin, 100 μL of the brain homogenates was mixed with 20 μL of 0.2% acetic acid containing 50% acetonitrile or atractylodin internal standard solution (Atractylenolide III, 200 μg mL⁻¹), followed by the addition of 250 μL of methanol, and then centrifuged (10,200 rpm, 10 minutes). The supernatants were loaded onto an Oasis HLB Elution Plate (Waters Corporation, Milford, MA, USA). In short, 350 μL of supernatant was applied, washed with 350 μL of purified water, and eluted with 100 μL of 0.2% acetic acid containing acetonitrile. The eluate was diluted with 50 μL of 0.2% acetic acid. Forty microliter of the solution was injected into the liquid chromatography coupled with tandem mass spectrometry system, comprising an Agilent 1260 series (Agilent Technologies) connected to a QTRAP5500 tandem mass spectrometer fitted with a TurboSpray electrospray ionization interface (AB Sciex Tokyo, Japan). A CAPCELL CORE ADME column (100×2.1 mm id, 2.7 μm; Shiseido, Tokyo, Japan) was used at 40°C. The mobile phase consisted of solution A (0.2% acetic acid) and solution B (0.2% acetic acid containing acetonitrile) with a gradient of solution B (64%, 5 minutes; 90%, 5-10 minutes; v/v) at a flow rate of 200 μL min⁻¹. The mass spectrometer was operated in the positive-ion mode. The high-purity nitrogen gas was composed of ion source gas 1, ion source gas 2, curtain gas, and collision-activated dissociation gas at pressures of 30, 0, 10, and 8 psi respectively. The optimized Turbo Spray voltage and temperature were set at 17 V and 600°C respectively. Atractylodin was quantified by multiple reaction monitoring transitions at 182.985 to 153.0 m z⁻¹.

Brain pharmacokinetic data were analyzed by non-compartmental modeling using Phoenix WinNonlin (version 6.4, Certara L.P., St. Louis, MO, USA) to determine Cₘ₃ₐp tₘ₃ₐ and AUCₙₗₒₜ.

2.8 | Statistical analysis

Animals were randomly allocated to experimental groups and sample size was based on preliminary experiments. In the
motility index examination, values for individual groups are shown as means±standard error (SE). To assess differences in motor activity among groups, a Student’s t test or a Tukey-Kramer test were performed. Plasma and brain concentration data from the pharmacokinetic analysis are presented as mean±SE. As for the plasma pharmacokinetic parameters, an analysis of variance (ANOVA) for log-transformed data ($C_{\text{max}}$ and $\text{AUC}_{\text{last}}$) or a Wilcoxon rank sum test for $t_{\text{max}}$ was used. Values of $P<.05$ were considered statistically significant.

3 | RESULTS

3.1 Effects of rikkunshito and atractylodin on ghrelin-induced gastric motility

In fasted rats, the gastric motility was observed to consist of cyclic changes in contraction waves, which included a quiescent period (phase I-like contractions) followed by a group of contractions (phase III-like contractions), as reported in our previous study. When ghrelin (3 nmol) was intravenously administered through the vessel catheter to fasted rats in a quiescent period, phase III-like contractions were immediately induced and maintained for 30 minutes (Figure 1A). The gastric motilities in fasted rats administered distilled water and rikkunshito 30 minutes before inducing phase III-like contractions by a second ghrelin administration are shown in Figures 1A and 1B. The %MI in the antrum was significantly increased by the administration of rikkunshito during fasting ($P<.05$, Figure 2). Rats given test meal (1 mL rat$^{-1}$)-gavaged rats exhibited fed motor activities in the antrum for about 30 minutes, followed by fasted motor patterns (C). At 30 minutes after ingestion of a test meal, DW (D) or RKT (1000 mg kg$^{-1}$, E) was administered to rats and the second ghrelin (3 nmol, iv.)-induced gastric motility was compared with the first one.

FIGURE 1 Influence of rikkunshito on ghrelin-induced gastric motility in rats. (A, B) Gastric motor activity of fasted rats was observed for 30 minutes after the first administration of ghrelin (3 nmol, iv.). Gastric motility for 30 minutes after the second administration of ghrelin in rats pretreated with distilled water (DW, A) or rikkunshito (RKT; 1000 mg kg$^{-1}$, po., B) was compared with that after the first administration of ghrelin. (C-E) Test meal (1 mL rat$^{-1}$)-gavaged rats exhibited fed motor activities in the antrum for 30 minutes onward (Figure 1C). The phase III-like contractions were induced by ghrelin administration in the postprandial phase (Figure 1D) as reported in previous studies. However, there was no significant difference in %MI following ghrelin administration between the fasted and postprandial phase ($P=.98$, Figure 2). Neither distilled water (Figure 1D) nor rikkunshito (Figure 1E) administration at 30 minutes after intragastric administration of a test meal influenced the ghrelin-induced phase III-like contractions. Under these conditions, no significant difference was observed between rikkunshito and distilled water in the %MI in the antrum ($P=.21$, Figure 2). The effect of the oral administration of atractylodin (1 mg kg$^{-1}$), an active ingredient of rikkunshito, to fasted rats on the phase III-like
To determine the distribution of atractylodin in the brain, brain samples were collected by decapitation from fasted and free-fed rats. Atractylodin was detected in the brains of both fasted and free-fed rats. But the concentration increased more rapidly in fasted than in free-fed rats, and a significant difference was observed at 0.25 hour following rikkunshito administration, which was the $t_{\text{max}}$ of plasma atractylodin in the fasted rats (fasted, $28.2\pm1.0$ vs free-fed, $7.5\pm1.1$ ng g$^{-1}$ brain; $P<.001$; Figure 5). Table 2 shows the pharmacokinetic parameters of brain atractylodin.

### 4 | DISCUSSION

In this study, we showed that the administration of rikkunshito and its active ingredient atractylodin to rats potentiates the phase III-like contractions induced by ghrelin treatment. This pharmacological effect of rikkunshito was more remarkable in fasted rats than that in rats administered a test meal. Moreover, we showed that atractylodin increases immediately in the plasma and brain of fasted rats, as compared to free-fed rats, after oral administration of rikkunshito. These results suggest that maintaining appropriate levels of its active ingredient atractylodin is important for eliciting a significant effect of rikkunshito, and that food in the stomach influences the effect of rikkunshito by delaying the absorption of atractylodin.

It is well known that ghrelin is involved in motor activities of the upper GI tract in physiologically fasted subjects$^{15}$. Phase III-like contractions in the gastroduodenum, which are fasted motor activities, are induced by endogenous ghrelin secretion$^{17,19}$. In this study, ghrelin administration immediately induced the phase III-like contractions both in the fasted and fed rats, which is consistent with previous studies$^{17,18}$. However, the difference in magnitudes between the fasted and postprandial phases remains unclear. In this study, because no significant difference in %MI was observed between both phases following ghrelin administration, it is considered that same degree of effect of ghrelin on the phase III-like contractions was observed between both phases within the doses or timings of ghrelin administration used. Rikkunshito has been proven to increase ghrelin/GHS-R binding activity and enhance the increase in cytosolic calcium ions elicited by ghrelin in GHS-R-expressing cells$^{10}$. In the present study, the ghrelin-induced phase III-like contractions were potentiated in fasted rats by the administration of rikkunshito. The potentiating effect of rikkunshito on ghrelin reactivity in animal models with gastric dysmotility was observed in our previous report$^{20}$. These findings suggest that these motor effects of rikkunshito are related to the increase in ghrelin receptor sensitization$^{10,20}$. Rikkunshito stimulates ghrelin secretion, and it is possible that a negative feedback mechanism of ghrelin secretion suppresses the phase III contraction induced by exogenous ghrelin administration. However, our results suggest that any negative influence is overcome by the activation of the ghrelin receptor by rikkunshito.

Atractylodin is the active ingredient predominantly detected in the plasma of healthy volunteers in a clinical pharmacokinetic study of rikkunshito$^{15}$. The administration of rikkunshito to fasted rats immediately increased the atractylodin concentration in plasma, and the maximum concentration was reached at 15 minutes in this study. The
administration of atractylodin also potentiated the gastric motility in rats at 30 minutes, which is almost completely consistent with the time till reaching peak levels of atractylodin in plasma after the administration of rikkunshito. Atractylodin has also been demonstrated to possess an enhancing effect on ghrelin receptor signaling. Therefore, it seems likely that the augmentation of ghrelin-induced motility in the antrum after the administration of rikkunshito is mediated by an increase in ghrelin receptor signaling by the active ingredient atractylodin.

The motor effect of rikkunshito was more significant in rats administered with it during fasting than after test meal ingestion by gavage. Food in the stomach is known to influence the bioavailability of various medicines. Therefore, a pharmacokinetic study of rikkunshito in free-fed and fasted rats was conducted. The administration of rikkunshito under the fasted condition significantly shortened $t_{\text{max}}$ and increased $C_{\text{max}}$ of atractylodin compared to that under the fed condition. It is common for the rate of absorption of drugs to be slower when taken with meals than during fasting because of decreased gastric emptying. According to our previous reports, atractylodin could not be absorbed through the stomach. Therefore, it is suggested that food in the stomach suppressed the increased plasma concentration of atractylodin. The
Atractylodin carboxylate, a metabolite of atractylodin, could be identified in the plasma after the administration of rikkunshito in a study of healthy humans. Like atractylodin, the metabolite increased the ghrelin/GHS-R binding activity in GSH-R-expressing cells, suggesting that it contributes to the ghrelin signal-enhancing activity of rikkunshito. However, the details of this action in vivo remain unclear.

Whether the metabolite of atractylodin is involved in the gastric motor effect of rikkunshito should be determined in a future study.

In conclusion, we have shown that the potentiating effect of the Kampo medicine rikkunshito on ghrelin-induced phase III-like contractions can be observed in fasted rats. The efficacy of rikkunshito may be associated with a decreased absorption rate of its active ingredient atractylodin when food is in the stomach. Rikkunshito has several clinical effects on postprandial symptoms. Rikkunshito administration before meal may be more effective in alleviating the onset of postprandial symptoms in patients.

DISCLOSURE

M. Nahata, Y. Mizuhara, C. Sadakane, J. Watanabe, N. Fujitsuka, and T. Hattori are employed by Tsumura & Co.

AUTHOR CONTRIBUTIONS

MN performed the research, analyzed the data, and drafted the manuscript. YM and CS performed the research and analyzed the data. JW supervised. NF designed the research study, performed the research, analyzed the data, and drafted the manuscript. TH analyzed and reviewed the data, and supervised.

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