POTENTIALS OF KAMPO MEDICINE IN MODERN SOCIETY

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POTENTIALS OF KAMPO MEDICINE IN MODERN SOCIETY

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Editorial: Potentials of Kampo Medicine in Modern Society

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Keywords: traditional Japanese medicine, Kampo, syakuyakukanzoto, keishibukuryogan, hochuekkito

Editorial on the Research Topic

Potentials of Kampo Medicine in Modern Society

Public education of Traditional Japanese (Kampo) Medicine in Japan was abruptly stopped in 1895. As a result, Kampo went into steep decline. Nevertheless, Kampo medicine has gradually reemerged and now occupies a considerable position in the field of medical practice and education (1, 2). Its unforeseen significant effect is occasionally observed in patients with intractable diseases that western-style medicines don't work at all (3). Moreover, the Kampo concept of tonifying is used in persons with frailty or intractable infections such as *MRSA* or multi-drug-resistant *Pseudomonas aeruginosa* (4, 5).

It is also applicable in both emergency and intensive care units (6). For example, shakuyakukanzoto enables the rapid control of myalgia and is used to treat tetanus-induced convulsions (7). Goreisan is used for fluid^{TM1} disturbance and for the treatment of vertigo and acute gastroenteritis (8). Furthermore, blood ^{TM1} disturbance is effective for the acute treatment of trauma. Hematoma is considered a form of static blood ^{TM1}; therefore, formulations that are useful for treating static blood ^{TM1}, such as keishibukuryogan and jidabokuippo, can be used (9–11). Satoh and Nakae reported that the administration of daijokito (DJT), which is composed of Magnolia bark, immature orange, rhubarb rhizome, and anhydrous mirabilitum, caused defecation in critically ill patients and significantly increased the stool volume. The anhydrous mirabilitum in DJT has a stool softening effect, and rhubarb rhizome has a hypermotility effect, and they are traditionally utilized couplings. Furthermore, Magnolia bark has psychotropic effects, and immature orange has anti-inflammatory effects. Such synergistic effects and multifunctionality are the strong points of Kampo medicine. While the negative effects of polypharmacy may occur to cover various effects with western medicines, the combination of crude drugs in Kampo medicine has been sophisticated throughout history.

In a medical environment that favors modern Western medicine, treatment with Kampo medicine is not common in emergency and critical care medicine. Nevertheless, treatments for acute infection, poisoning, or resuscitation are described in Shanghan Lun and Jin Gui Yao Lue, regarded as "emergency manuals," both written by Zhang Zhongjing (150–219). We should apply such manuals as a gift of wisdom from ancestors and use them as suitable for our modern society.

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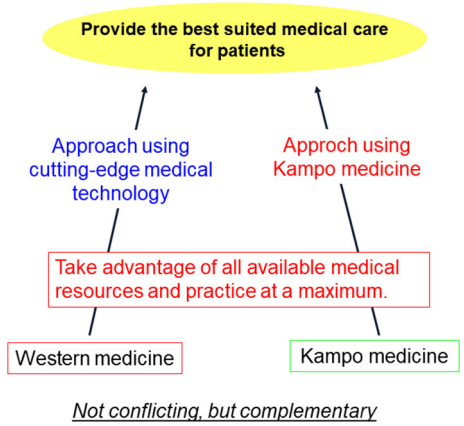

FIGURE 1 | Hybrid-type treatment using Western and Kampo medicine.

Kampo medicine might be applied to coronavirus disease 2019 (COVID-19) as well, since it has been used for viral infections such as influenza (12). Heat-clearing formula such as saikatsugekito is expected to prevent serious illness in mild cases (13–17). Tonic formula such as hochuekkito may prevent infections since it has multiple effects through the digestive and immune systems, including for acute viral infection and chronic inflammation (18, 19).

Thus, the quality of acute and chronic treatment strategies may be improved by taking advantage of all available medical resources and practices such as Western and Kampo medicines (**Figure 1**).

Some adverse events that may present a risk of occurring in patients based on the known actions of the major active components of certain drugs are as follows; Ephedra herb, Glycyrrhiza root, Aconite tuber, rhubarb rhizome, and anhydrous mirabilitum (20). Yoshino et al. summarized clinical risk factors of Licorice-induced pseudoaldosteronism in this topic.

Now is the time to recognize Kampo medicine is effective in a variety of medical areas in a modern society.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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CASE REPORT
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Kampo Medicine Treatment for Advanced Pancreatic Cancer: A Case Series

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Aims: The present report aims to investigate the use of Kampo medicine for advanced pancreatic cancer patients in order to prolong survival.

Methods: We retrospectively reviewed medical records of patients with pancreatic cancer who presented to our Shimizu Clinic from 2000 to 2020. Patients who survived at least twice as long as the initial prognostic estimate were selected and their treatment was reviewed. The Kampo formula and crude drugs were selected according to the Kampo diagnosis and treatment strategy, which included qi and blood supplementation; qi, blood and water smoothing; and inflammation (termed "heat") and cancer suppression.

Results: Ten patients aged 45–80 years (six males and four females) with stage IV advanced cancer were selected. All patients received hozai, which is a tonic formula, of juzentaihoto (JTT) or hochuekkito (HET) decoction. Anti-cancer crude drugs were included in the decoctions of nine patients. At the first visit, the estimated life expectancy for all patients was no more than 1 year; however, treatment with Western and Kampo medicine led to a relatively long survival period of over 2 years. Three patients were still living at the time of this writing, more than 2, 6, and 14 years after treatment initiation.

Conclusion: Our results suggest that Kampo medicine may be useful for disease control and supportive care for patients with advanced pancreatic cancer.

Keywords: pancreatic cancer, Kampo medicine, integrative therapy, quality of life, prolong survival

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INTRODUCTION

Surgery, radiotherapy, and chemotherapy along with anticancer drugs are the standard Western treatments for pancreatic cancer. However, the therapeutic effects of these treatments are poor in cases of advanced disease, with a 5-year survival rate of 1.3% among patients with advanced pancreatic cancer (1). For patients with advanced cancer who have not responded to Western medicine, an integrated treatment approach using Kampo medicine may be a useful alternative. Kampo medicine is beneficial for the treatment of cancer-related numbness, constipation, anorexia, muscle cramps, and fatigue (2). Given this finding, the Japanese Society for Palliative Medicine has recommended the use of Kampo medicine and crude drugs in combination with Western medicine (3). Improvement of symptoms during cancer treatment may extend the patients' tolerance for longer treatment periods (4, 5).

We previously presented a case of a patient with advanced pancreatic cancer who survived for 7 years after diagnosis without a significant decrease in quality of life (QOL) (6). This initial case demonstrated that Kampo medicine may be useful for disease control and supportive care for patients with advanced pancreatic cancer. To study this idea further, we retrospectively reviewed cases of advanced pancreatic cancer seen in our clinic to summarise and evaluate the efficacy of Kampo treatment for this patient group.

METHODS

Medical records of 48 patients with advanced pancreatic cancer who were treated with Kampo medicine in our Shimizu Clinic from the year 2000 to the year 2020 were included in this retrospective review. Ultimately, 10 patients who lived at least twice as long as the estimated prognosis, determined at the time of the first visit, were enrolled for the study. The patients' age, sex, clinical stage of disease at the first visit, life expectancy at the first visit, Western medicine treatment, Kampo medicine treatment, and survival duration were collected from their medical records. The Kampo formula and crude drugs were selected according to the Kampo diagnosis and treatment strategy. This strategy entailed qi and blood supplementation; qi, blood and water smoothing; and inflammation (heat) and cancer suppression. The Kampo formulas of hozai and kuoketsuzai and anti-cancer drugs used for the patients are listed in **Table 1**. The concentration indicator and composition of each crude drug are regulated by Japanese Pharmacopoeia of Japan version 17th.

Ethical Considerations

This case series was approved by the Institutional Review Board of the Tohoku University Graduate School of Medicine (Institutional Review Board No. 18910).

RESULTS

Ten patients between the ages of 45 and 80 years (six males and four females) with stage IV advanced cancer were selected (**Table 2**). All Kampo formulas were prescribed as decoctions. All patients received hozai, which is a tonic formula of juzentaihoto (JTT) or hochuekkito (HET). Anti-cancer crude drugs such as Hedyotis diffusa, Scutellaria barbata, and/or Lobelia chinensis were administered to patients 1 through 10, with the exception of patient 9. Patients 1, 2, and 8 received additional Kampo medicine for the relief of chemotherapy-related symptoms. At the first visit, the life expectancy of all patients was limited to no more than 1 year; however, treatment with Western medicine and Kampo medicine led to a relatively long survival period of over 2 years. Three patients undergoing Kampo treatment were still living at the time of this writing, more than 2, 6, and 14 years after treatment initiation.

DISCUSSION

The cases presented herein indicate that Kampo medicine can be provided for advanced pancreatic cancer treatment as an integrative cancer therapy. Additionally, these cases suggest that Kampo medicine may slow cancer progression and improve the survival rate. The treatment strategy for advanced cancer patients includes supporting vital energy and nutrition, harmonising

TABLE 1 | Kampo formula, its constituents, and mechanisms according to prior studies.

Kampo formula	Crude drug	Additional mechanisms for cancer and immune system according to prior studies
Juzentaihoto (JTT)	Astragalus root, cinnamon bark, rehmannia root, peony root, cnidium rhizome, atractylodes lancea rhizome, angelica root, ginseng, poria sclerotium, glycyrrhiza	Prevention of malignant progression and tumour cell metastasis (7). Upregulation of T cell activities by decreasing Foxp3 (+) Treg populations (8). Enhancement of fluorouracil-induced myelosuppression (9).
Hochuekkito (HET)	Astragalus root, atractylodes lancea rhizome, ginseng, angelica root, bupleurum root, jujube, citrus unshiu peel, glycyrrhiza, cimicifuga rhizome, ginger	Enhancement of concomitant immunity against tumour development (10). Restoration of antitumor T cell responses by normalisation of serum corticosterone, interleukin (IL)-12, and costimulatory molecule expression (11). Maintenance of NK cell activity and suppression of stress mediators (12). Inhibition of proinflammatory cytokine production, particularly IL-6 (13). Enhancement of cisplatin-induced apoptosis (14). Inhibition of cytokine-mediated apoptosis or necrosis, resulting in a reduction of the gastrointestinal side-effects of cancer chemotherapy (15). B cell replenishment after radiotherapy (16).
Keppuchikuoto	Angelica root, peony root, cnidium rhizome, rehmannia root, bupleurum root, glycyrrhiza, peach kernel, platycodon root, safflower, achyranthes root, immature orange	Stimulation of IL-2 and tumor necrosis factor (TNF)-α secretion and enhancement of their immune function, resulting in tumour growth suppression (17).
	Hedyotis diffusa	Anticancer, antitoxic, and diuretic effects (18).
	Scutellaria barbata	Inhibition of growth several human cancers, including lung cancer, digestive system cancers, hepatoma, breast cancer, and chorioepithelioma (19).
	Lobelia chinensis	Inhibition of inducible nitric oxide synthase, cyclooxygenase-2, TNF-α, and IL-6 from the NF-κB pathway (20).

TABLE 2 | Patients with advanced pancreatic cancer who were treated with Kampo medicine and lived at least twice as long as the prognosis determined at the time of initial visit.

Case number	Age (years)	Sex	Disease	Onset	Diagnosis method	Day of surgery	Surgery	Anti-cancer drug/radiation	First visit at clinic	Performance status at first visit	Stage at the first visit	Life expectancy at the first visit	Western medicine treatment	Kampo medicine treatment	Tumour marker trend	Survival	Current status
1	45	M	Pancreatic cancer adenocarcinoma invasive ductal carcinoma, tub2	01/05/2003	Operation	11/06/2003	Distal pancreatectomy	1. Gemcitabine hydrochloride 2. Tegafur, gimeracil, oteracil potassium	25/08/2003	1	IVa	1 year	Surgery for primary tumour and lung metastases; chemotherapy; radiotherapy for brain metastases	JTT† and Keppuchikuoto with *Hedyotis diffusa, Scutellaria barbata, Lobelia chinensis*, and Fructus Polygoni Orientalis for suppression of cancer and support of physical strength; Senpukuka-taishasekito, Goreisan, Corydails Tuber for symptoms of nausea, vomiting, headache, and vertigo.	Gradual increase	7 years	Death due to primary disease
2	74	F	Pancreatic cancer	N/A	CT	N/A	N/A	Gemcitabine hydrochloride	09/05/2006	3	IVb	3 months	Chemotherapy	JTT with *H. diffusa* for suppression of cancer and support of physical strength; Senpukuka-taishasekito for symptoms of nausea and vomiting.	Gradual decrease	3 years	Death
3	67	M	Pancreatic cancer, Gastric cancer, Thyroid cancer	N/A	Operation	26/11/2014	Distal pancreatectomy	Radiation	31/01/2015	2	IVb	1 year	Surgery for primary tumour and liver metastases	HET‡ with *H. diffusa* and Fructus Polygoni Orientalis for suppression of cancer and support of physical strength.	Gradual decrease	>6 years	Still alive
4	72	M	Pancreatic cancer	17/03/2013	Operation	8/4/2013, 9/8/2013	Distal pancreatectomy, Pancreatectomy	Tegafur, gimeracil, oteracil potassium	04/07/2013	2	IVa	1 year	Surgery for primary tumour and bile duct metastases	HET with *H. diffusa* and Fructus Polygoni Orientalis for suppression of cancer and support of physical strength; Inchinkoto for its cholagogic effect.	Gradual decrease	3 years	Death due to interstitial pneumonia
5	73	M	Pancreatic cancer, Lung cancer, Tongue cancer	01/09/1991	Operation	01/10/2006	Pancreatoduodenectomy	Gemcitabine hydrochloride	12/10/2006	1	IVb	1 year	Surgery for primary tumour; chemotherapy	HET with *H. diffusa* for suppression of cancer and support of physical strength; Inchinkoto for it cholagogic effect.	Gradual decrease	>14 years	Death due to pneumonia
6	61	M	Pancreatic cancer	01/12/2008	CT	N/A	N/A	Gemcitabine hydrochloride	20/07/2017	2	IVb	6 months	Chemotherapy	HET with *H. diffusa* for suppression of cancer and support of physical strength.	Gradual increase	6 years	Death
7	68	F	Pancreatic cancer	04/04/2015	CT	15/10/2015	Pancreatic head resection	Gemcitabine hydrochloride	20/01/2019	2	IVb	1 year	Surgery for primary tumour; chemotherapy	HET with *H. diffusa* for suppression of cancer and support of physical strength.	Gradual increase	>2 years	Still alive
8	80	F	Pancreatic cancer	16/05/2017	CT	01/06/2017	Primary inoperable, bile duct stenting	Gemcitabine hydrochloride	13/06/2017	2	IVb	6 months	Surgery for primary tumour; chemotherapy	JTT with *S. barbata* and Fructus Polygoni Orientalis for suppression of cancer and support of physical strength. Senpukuka-taishasekito for symptoms of nausea and vomiting.	Gradual decrease	3 years	Death
9	62	M	Pancreatic cancer	01/02/2017	MRCP	N/A	Inoperable	1. Paclitaxel 2. Gemcitabine hydrochloride	27/04/2017	1	IVb	6 months	Chemotherapy	HET for support of physical strength.	Gradual increase	3 years	Death
10	78	F	Pancreatic cancer	25/05/2016	MRI	N/A	Inoperable	N/A	18/06/2016	2	IVb	1 year	Palliative care only	HET with *H. diffusa* and *S. barbata* for suppression of cancer and support of physical strength.	Gradual increase	3 years	Death

N/A, not assigned; CT, Computed Tomography; MRCP, Magnetic Resonance cholangiopancreatography; MRI, Magnetic Resonance Imaging. †*JTT, Juzentaihoto;* ‡*HET, Hocuekkito.*

the immune system and sympathetic condition, suppressing inflammation, and promoting microcirculation and interstitial fluid. These concepts are expressed as balancing qi, blood, and fluid or cold and heat within Kampo theory. Anti-cancer drugs attack cancer cells but also cause body damage, which reduces body recovery and innate immunity. On the other hand, Kampo medicines act on biological reactions and they have a supplementary effect on recovery and reduced immune system. This characteristic is important for striking a balance between offence against cancer and defence for the whole body.

Hozai, such as JTT or HET, are used to support vital energy and nutrition and to harmonise the immune system and sympathetic conditions. The indications for JTT include declined constitution after recovery, fatigue and malaise, anorexia, and anaemia. Additional effects of JTT on suppression of cancer growth, including prevention of malignant progression and tumour cell metastasis (7), upregulation of T cell activity (8), and improving fluorouracil-induced myelosuppression (9) have been reported in several experimental studies. HET has been reported to reduce cancer-related fatigue and improve QOL for cancer patients (21, 22). Additional reported effects of HET on cancer include concomitant enhancement of immunity against tumour development (10); restoration of antitumor T cell responses, and costimulatory molecule expression (11); maintenance of NK cell activity and inhibition of stress mediators (12); inhibition of proinflammatory cytokine production (13); enhancement of cisplatin-induced apoptosis (14); and inhibition of cytokine-mediated apoptosis or necrosis, leading to a reduction of the gastrointestinal side effects of cancer chemotherapy (15); and replenishing B cells after radiotherapy (16). These reports support the possibility of suppressing tumour growth and affecting immunomodulation to reduce inflammation in addition to the original supportive effects for fatigue and malaise. HET also can promote negative conversion of vancomycin-resistant Enterococci or prevent the colonisation of methicillin-resistant Staphylococcus aureus in humans (23, 24). These studies suggested that HET influenced on innate immunity and nutrition status.

Kuoketsuzai, which is a blood stasis-resolving formula such as Keppuchikuoto, has been used for relief of pain caused by blood stasis (25). It stimulates interleukin (IL)-2 and tumor necrosis factor (TNF)-α secretion and improves immune function, resulting in tumour growth suppression (17). For pain control, some crude drugs were added to the formula. Corydalis tuber, a crude drug that contains isoquinoline alkaloids, is used for intractable pain and can be used to manage pain associated with bone metastases.

Some herbal medicines may be added to Kampo treatment due to their anti-cancer effects. *H. diffusa* is used as an anticancer, antitoxic, and diuretic agent to treat cancers (18). Extracts of *S. barbata* have inhibitory effects on the growth of several cancers in humans, including lung cancer, gastrointestinal cancers, hepatoma, breast cancer, and chorioepithelioma (19). *L. chinensis* has anti-inflammatory properties that are attributable to inhibition of inducible nitric oxide synthase, cyclooxygenase-2, TNF-α, and IL-6 *via* the NF-κB pathway (20). The combination of hozai with anti-cancer crude drugs may support patient condition and inhibit cancer growth, resulting QOL and relatively prolonged survival rate.

In 2019, the 11th revision of the International Statistical Classification of Diseases and Related Health Problems published by the World Health Organisation included a traditional medicine module (26). Following this global trend, recently, most of the clinical practice guidelines in Japan recommended Kampo medicines for symptoms and diseases (27–29). Our previous report suggested that integrative medicine combined with Kampo medicine and western medicine can be applied for several intractable symptoms and diseases (6, 30–34). Therefore, Kampo treatment may be a helpful tool during advanced pancreatic cancer treatment.

Nowadays, the term "integrative oncology" is used for multidisciplinary cancer treatment. It includes a combination of complementary medicine in conjunction with conventional cancer treatments (35). Complementary medicine and traditional medicine have been incorporated to the contents of the Basic Medical Education: Japanese Specifications WFME (World Federation for Medical Education) Global Standards for Quality Improvement (36). It showed to have an opportunity to contact complementary medicine and traditional medicine in Japan. Furthermore, Model Core Curriculum for Medical Education revised at 2017 included the objectives of outlining the characteristics of Kampo medicine, the indications and pharmacological effects of major Kampo medicines (37). Considering these concepts and educational process, it is important to understand the characteristics of Kampo medicines and use them effectively along with conventional cancer treatments.

This research has some limitations. The study design is a case series; we did not include control or comparison groups. In the retrospective analysis, we did not have complete data of patients' entire clinical course. Thus, we could not compare the factors relating to prognosis between delayed prognosis and poor prognosis. Further, study will be needed to clarify the factors relating to prognosis including Kampo treatment.

In conclusion, Kampo medicine may be useful for disease control and supportive care for patients with advanced pancreatic cancer and may result in a relatively prolonged survival rate.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This case series was approved by the Institutional Review Board of the Tohoku University Graduate

School of Medicine (Institutional Review Board No. 18910). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MS treated patients. MS and ST wrote manuscript. AK, RO, and RA selected patients from medical records. KI and TI revised manuscript. All authors contributed to the article and approved the submitted version.

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Clinical Risk Factors of Licorice-Induced Pseudoaldosteronism Based on Glycyrrhizin-Metabolite Concentrations: A Narrative Review

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Licorice, the dried root or stolon of *Glycyrrhiza glabra* or *G. ularensis*, is commonly used worldwide as a food sweetener or crude drug. Its major ingredient is glycyrrhizin. Hypokalemia or pseudoaldosteronism (PsA) is one of the most frequent side effects of licorice intake. Glycyrrhizin metabolites inhibit type 2 11β-hydroxysteroid dehydrogenase (11βHSD2), which decomposes cortisol into inactive cortisone in the distal nephron, thereby inducing mineralocorticoid receptor activity. Among the several reported glycyrrhizin-metabolites, 18β-glycyrrhetyl-3-*O*-sulfate is the major compound found in humans after licorice consumption, followed by glycyrrhetinic acid. These metabolites are highly bound to albumin in blood circulation and are predominantly excreted into bile via multidrug resistance-associated protein 2 (Mrp2). High dosage and long-term use of licorice are constitutional risk factors for PsA. Orally administered glycyrrhizin is effectively hydrolyzed to glycyrrhetinic acid by the intestinal bacteria in constipated patients, which enhances the bioavailability of glycyrrhizin metabolites. Under hypoalbuminemic conditions, the unbound metabolite fractions can reach 11βHSD2 at the distal nephron. Hyper direct-bilirubin could be a surrogate marker of Mrp2 dysfunction, which results in metabolite accumulation. Older age is associated with reduced 11βHSD2 function, and several concomitant medications, such as diuretics, have been reported to affect the phenotype. This review summarizes several factors related to licorice-induced PsA, including daily dosage, long-term use, constipation, hypoalbuminemia, hyper direct-bilirubin, older age, and concomitant medications.

Keywords: Japanese Kampo medicine, licorice, pseudoaldosteronism, hypokalemia, pharmacokinetics

INTRODUCTION

Licorice, the dried root or stolon of *Glycyrrhiza glabra* or *G. ularensis*, is commonly used as a food sweetener or crude drug worldwide. Wide-range pharmaceutical effectiveness of licorice includes anti-ulcer, anti-spasmodic, anti-inflammatory, anti-oxidative, anti-virus, anti-microbial, anti-carcinogenic, and anti-androgenic properties (1). Pseudoaldosteronism (PsA) is, however,

one of the most frequent side effects of licorice intake and has been well described by Conn et al. (2). The clinical presentation of PsA is similar to that of primary aldosteronism and is characterized by peripheral edema, hypertension, laboratory hypokalemia, and lower plasma renin activity, due to the excessive action of mineralocorticoid receptors. The mineralocorticoid receptor stabilizes epithelial sodium channels on the apical side of the cortical collecting duct principal cell, which increases sodium reabsorption, corresponding to peripheral edema, hypertension, and lower plasma renin activity (**Figure 1**), whereas potassium is excreted as a cationic ion via the renal outer medullary potassium channel, which results in hypokalemia and, in severe cases, myopathy or arrhythmia (1). Most cases of licorice-induced PsA are self-limiting and are resolved once licorice intake ceases, without any specific treatment. However, some cases can progress to severe hypokalemia and life-threatening arrhythmia (3).

PATHOPHYSIOLOGY OF LICORICE-INDUCED PsA

Licorice contains glycyrrhizin (GL), a glycoside of glycyrrhetinic acid (GA) containing two molecules of glucuronic acid. Licorice has long been known to exert corticosteroid-like action, and GL and GA are considered to cause PsA by binding to mineralocorticoid receptors (2). However, their affinity for the receptor is significantly lower than that of the original substrate, aldosterone (4, 5), and the hypothesis that it acts by directly binding to the receptor at the actual blood concentrations of GL and GA is unrealistic (1). Furthermore, previous studies found that licorice-induced PsA did not occur in patients and animals with adrenal deficiency who had lower blood cortisol levels (6–10).

Cortisol, an adrenocortical hormone, has the same affinity for mineralocorticoid receptors as aldosterone; however, it occurs at a higher concentration in the blood. Cortisol is then decomposed by type 2 11β-hydroxysteroid dehydrogenase (11βHSD2) in renal tubule cell cytoplasm into cortisone, which has a lower affinity for the receptor (11), especially at the distal nephron (12, 13) in the normal state. Inhibition of 11βHSD2 by GL metabolites, rather than direct binding of GL or its metabolites to mineralocorticoid receptors was thus considered as the mechanism of licorice-induced PsA (14–16). With GL metabolites in the 11βHSD2-expressing cells, inhibition of 11βHSD2 results in a higher concentration of cortisol that binds to and stimulates the mineralocorticoid receptor (**Figure 1**). Therefore, the difference between primary aldosteronism and PsA is the plasma aldosterone concentration, which is lower in PsA and higher in primary aldosteronism. The aldosterone concentration is suppressed by negative feedback in licorice-induced PsA, even though aldosterone metabolism in the liver could be suppressed by GL or GA (17).

There are several other causes of PsA, including enzymatic defects in adrenal steroidogenesis (deficiency of 17α-hydroxylase and 11β-hydroxylase), gain-of-function mutations in the mineralocorticoid receptor (18), saturation of mineralocorticoid receptor binding by cortisol (Cushing syndrome), alterations in 11βHSD2 (syndrome of apparent mineralocorticoid excess), and genetic alterations in sodium channel expression (Liddle syndrome) or the sodium-chloride co-transporter (Gordon syndrome) (19).

GL-METABOLITES THAT TRULY REACH AND INHIBIT TYPE 2 11β-HYDROXYSTEROID DEHYDROGENASE

When GL is administered orally, it cannot be absorbed in its original form owing to its molecular structure, which contains both hydrophobic and hydrophilic parts. Orally ingested GL is hydrolyzed to GA by the intestinal bacteria (20–24), and GA appears as the main metabolite in blood circulation (20). Both GA and GL inhibit 11βHSD2 *in vitro*, but the inhibitory activity of GA is approximately 200 times higher than that of GL (15). Further, as GL does not appear in blood after licorice ingestion, GA has been considered as the causative agent for the onset of licorice-induced PsA that inhibits 11βHSD2 in humans. However, the plasma concentration of 3-monoglucuronyl glycyrrhetinic acid (3MGA) was reported to be significantly higher in patients with hypokalemia than in those with normokalemia and chronic hepatitis who had been orally treated with GL for more than four weeks, even though the plasma concentration of GA did not differ between the two groups (25). When GL is injected intravenously into rats, it is partially metabolized to 3MGA in the liver by lysosomal β-D-glucuronidase, after which GL and 3MGA are excreted with bile (26). Although 3MGA did not appear in the blood circulation and urine of normal Sprague-Dawley rats that were orally treated with GA, it was found in the blood and urine of multidrug resistance-associated protein (Mrp) 2-deficient Eisai hyperbilirubinuric rats (EHBR) (27). Both GA and 3MGA have high affinity for albumin (28–31); however, only 3MGA is the substrate of organic anion transporter (OAT) 1 and OAT3, which are expressed at the basolateral membrane of renal tubular epithelial cells (31). As a substrate of basolateral transporters, 3MGA is compatible with the intracellular space where 11βHSD2 is located, and is considered a causative agent of PsA. However, 3MGA has not been detected by mass spectrometry in humans after licorice intake (32–34). Instead, we isolated and identified 22α-hydroxy-18β-glycyrrhetyl-3-*O*-sulfate-30-glucuronide (compound 1), 22α-hydroxy-18β-glycyrrhetyl-3-*O*-sulfate (compound 2), 18β-glycyrrhetyl-3-*O*-sulfate (compound 3, GA3S), and 18β-glycyrrhetyl-3-*O*-sulfate-30-glucuronide (compound 4) as other GL metabolites in the urine of EHBR orally treated with GA (34–36). We also found that the blood and urinary concentrations of these new metabolites were much higher than those of 3MGA in EHBR orally treated with GA, and that their pharmacokinetic behavior was similar to that of 3MGA (34, 35). In humans, GA3S was detected at the highest concentration in the blood of patients with PsA who developed rhabdomyolysis due to licorice (34). Further, there were no cases where compound 1 was detected, while compound 2, GL, and 3MGA were rarely detected. The concentration of GA3S was still the highest, followed by GA and compound 4 in the serum of

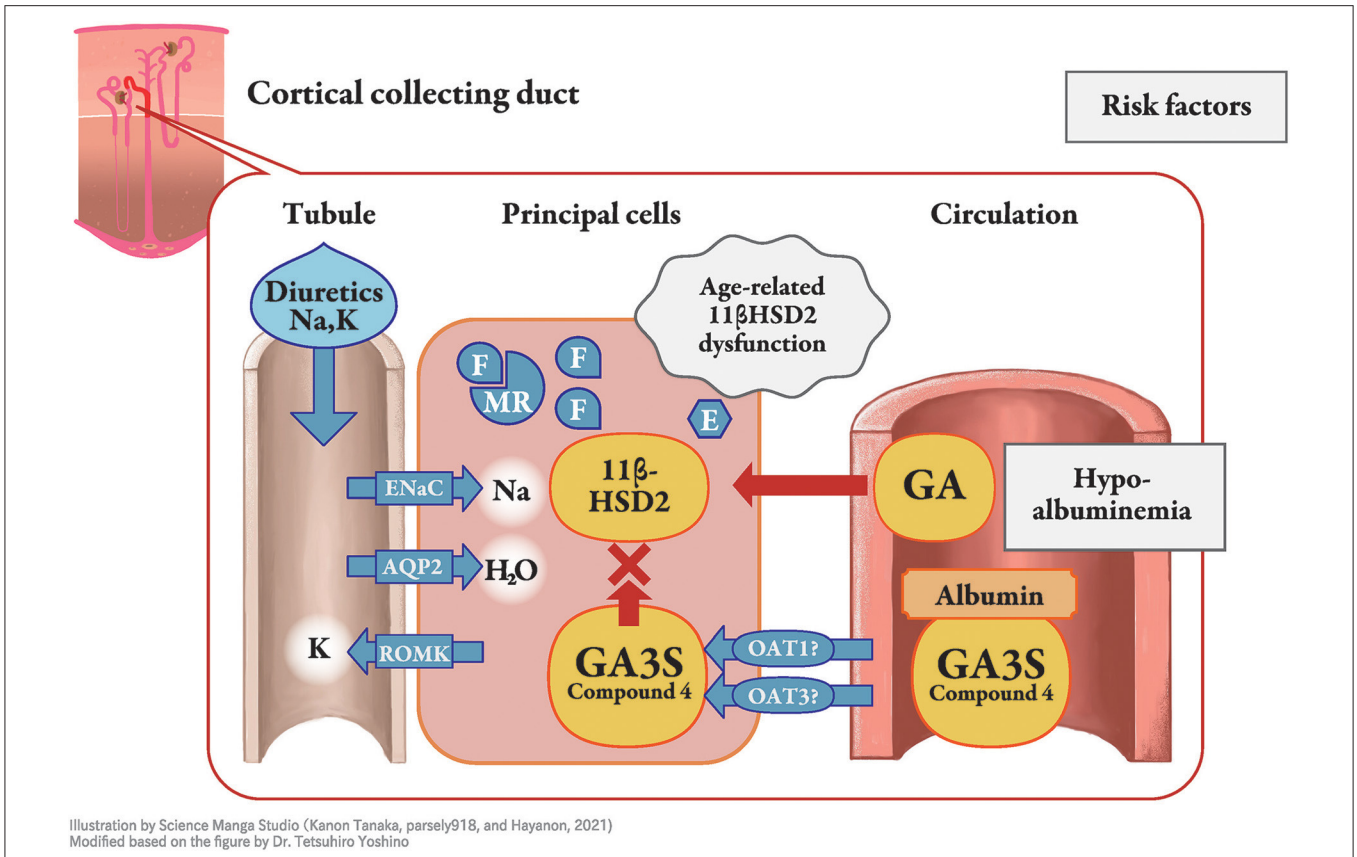

FIGURE 1 | Molecular mechanism of licorice-induced pseudoaldosteronism. At the distal nephron, especially in the principal cells of the cortical collecting duct, type 2 11β-hydroxysteroid dehydrogenase (11β-HSD2) is inhibited by glycyrrhetinic acid-3-*O*-sulphate (GA3S) and possibly other metabolites, resulting in increased levels of cortisol (F), with similar affinity to the mineralocorticoid receptor as that of for aldosterone. Mineralocorticoid receptors (MR) induce stabilization of epithelial sodium channels (ENaC) on the apical side, which increases sodium reabsorption. This results in peripheral edema, hypertension, and lower plasma renin activity, whereas potassium is excreted as a cationic ion via the renal outer medullary potassium channel. The unbound fraction of metabolites under hypoalbuminemia enables them to efficiently reach 11β-HSD2. Older age is related to lower function of 11β-HSD2, and diuretics affect their phenotype. E, cortisone; AQP, aquaporin; ROMK, renal outer medullary potassium channel; GA, glycyrrhetinic acid; OAT, organic anion transporter.

patients in a multicenter retrospective case series of milder PsA (33, 36). Therefore, we considered GA3S to be the most likely causative agent of PsA.

DIAGNOSIS OF LICORICE-INDUCED PsA

There is no concrete diagnostic criterion for licorice-induced PsA, and diagnosis is purely based on the clinical presentation of patients during licorice intake rather than by measuring the GL metabolite accumulation in blood. Therefore, diagnosing PsA is challenging, especially in mild cases. The serum concentrations of GA3S, GA, and compound 4 were found to be negatively correlated with serum potassium concentration, plasma renin activity, and aldosterone concentration (33, 36). From these results, it is suggested that such GL-metabolites could be used as an objective laboratory marker of "licorice-induced" PsA, and could be applied to the early detection and prevention of licorice-induced PsA especially in high risk patients.

As it is difficult to diagnose PsA based only on physical examination, including peripheral edema and hypertension, laboratory testing of parameters such as potassium and aldosterone levels is needed. The grade or occurrence of peripheral edema and hypertension in patients who consumed licorice did not correlate well with laboratory abnormalities, such as hypokalemia, low renin activity, aldosterone concentration, and GL metabolites (33, 36). Several studies have shown the expression of mineralocorticoid receptor and 11βHSD1/2 in the heart and arteries (37–39). Inhibition of 11βHSD2 resulted in an increased contractile response to phenylephrine (40) and altered endothelium-dependent relaxation of arteries due to decreased endothelial nitric oxide and increased endothelin-1 (41). These observations suggest that renal sodium reabsorption cannot fully explain hypertension in licorice-induced PsA.

CLINICAL RISK FACTORS FOR LICORICE-INDUCED PsA

Pharmacokinetics

Daily Dosage

The daily dosage of licorice is a reasonable risk factor for licorice-induced PsA (42, 43). Of the 147 oral Kampo medicinal products for ethical use in Japan, 109 (74%) contain extracts with 1.0–8.0 g licorice for daily dose. Their package inserts describe hypertension, hypokalemia, arrhythmia, and rhabdomyolysis

as adverse events of licorice-induced PsA. Kampo medicinal products containing more than or equal to 2.5 g licorice daily are contraindicated in patients with aldosteronism, myopathy, or hypokalemia, and those containing <2.5 g licorice daily still indicate these PsA-related conditions as potential side effects. As 74% of Kampo medicinal products contain GL, the use of multiple Kampo medicinal products can result in excessive GL ingestion, especially if the medicines are prescribed in multiple hospitals and clinics simultaneously.

Long-Term Use

Long-term use (>30 days) is also an important factor for developing licorice-induced PsA and related symptoms. In a retrospective cohort study, more than 80% of patients developed licorice-induced PsA and related symptoms, including hypokalemia in elderly patients (>60 years old) who received shakuyakukanzoto, which contains 6.0 g licorice, for longer than 30 days (44). This observation in the study is reasonable as the inhibition of 11βHSD plateaued after 2 weeks of GL ingestion (45).

Absorption

Constipation

When GL is administered orally, it is hydrolyzed to GA by the intestinal bacteria (20) and then absorbed into blood (**Figure 2**). GA3S, a sulfate conjugate of GA which is excreted via bile, is also hydrolyzed to GA by the intestinal bacteria and is then partially absorbed from the intestine into the blood circulation to exhibit enterohepatic circulation. The unabsorbed portions of GA and GA3S are then excreted in the feces. Thus, large individual variations in the blood concentration of GA were found even at the same dosage of GL or licorice. This variation may be due to the difference in the activity of hydrolyzed GL in the intestinal bacterial flora among the subjects. The hydrolyzation ratio also depends on the residence time of GL in the intestinal tract. The longer GL stays in the intestinal tract, the more it is hydrolyzed by bacterial β-glucuronidase, resulting in a higher serum concentration of GA (46).

Distribution

As GL metabolites are highly bound to serum albumin (>99.9%) in blood circulation (47, 48), they are not excreted in urine through glomerular filtration. However, they can be transported from blood circulation into tubular cells via OAT1 and OAT3, or other transporters and can participate in the inhibition of intracellular 11βHSD2 as well as their unbound forms (33–35). Hypoalbuminemia increases the unbound fraction of metabolites in blood circulation (30), resulting in enhanced distribution into principal cells where 11β-HSD2 is located (**Figure 1**). Therefore, hypoalbuminemia may be an important risk factor for PsA. Indeed, hypoalbuminemia has been identified as a risk factor in patients receiving yokukansan, which contains 1.5 g of licorice daily (49), as well as in other case series (50, 51).

Metabolism

To the best of our knowledge, there are no reports describing an individual variety of primary or secondary metabolism of GA in humans. We have previously reported that GA is conjugated by human liver sulfotransferase 2A1 (dehydroepiandrosterone sulfotransferase) into GA3S at C-3 (**Figure 2**). As serum concentrations of GA and GA3S correlated well, regardless of sex, sulfate conjugation is suggested to have a limited impact on inter-individual variation in GA pharmacokinetics (33).

Excretion

When bile excretion of GA3S is suppressed due to Mrp2 dysfunction (**Figure 2**), GA3S is transferred into the blood circulation. We have previously highlighted the involvement of Mrp2 in the biliary excretion of GL metabolites in rats (27, 34, 35) and possibly in humans (50, 51). GL metabolites significantly accumulate in the EHBR (27, 34, 35). While this phenomenon is still controversial in humans, we reported the co-occurrence of hypokalemia and hyperbilirubinemia, which could be a surrogate marker for MRP2 dysfunction in several cases; further, hypokalemia was found to be more common in patients with hyperbilirubinemia (50, 51). We consider hyper direct-bilirubin as a rare risk factor for licorice-induced PsA when compared with hypoalbuminemia, which is more common in outpatient settings (51).

Other Factors

Age

Older age may affect several processes of GL metabolism, and licorice-induced PsA is common in elderly patients (43, 52). As age-dependent decrease in 11 βHSD2 activity in hypertensive patients has been reported (53), alteration in enzyme activity in aging is another risk factor for elderly subjects (**Figure 1**). In addition, elevation of serum cortisol concentrations occur in elderly which may be associated with decreased negative feedback at the hippocampus related to decreased glucocorticoid receptor concentration (54). This phenomenon could result in the inhibition of 11βHSD2 and also explains why licorice-induced PsA is common among elderly patients. To the best of our knowledge, there is no case report of licorice-induced PsA in pediatric patients meaning that licorice and GL is safe to use in children.

Female Sex

Several other factors have been proposed as risk factors for licorice-induced PsA, including female sex (55, 56), lower body weight (43), and reduced body surface area (57), which have not been well explained and might be confounding factors (52). Theoretically, constipation is common in female patients, and the higher dosage administered despite lower body weight and surface area compared with that in male patients even with the same daily dosage of licorice.

Concomitant Use of Medications

Concomitant use of medications, including antihypertensives, especially thiazide, loop diuretics, aldosterone blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers, affects the phenotype of PsA. For example, concomitant use of potassium-losing diuretics increases the risk of hypokalemia in patients treated with yokukansan, an

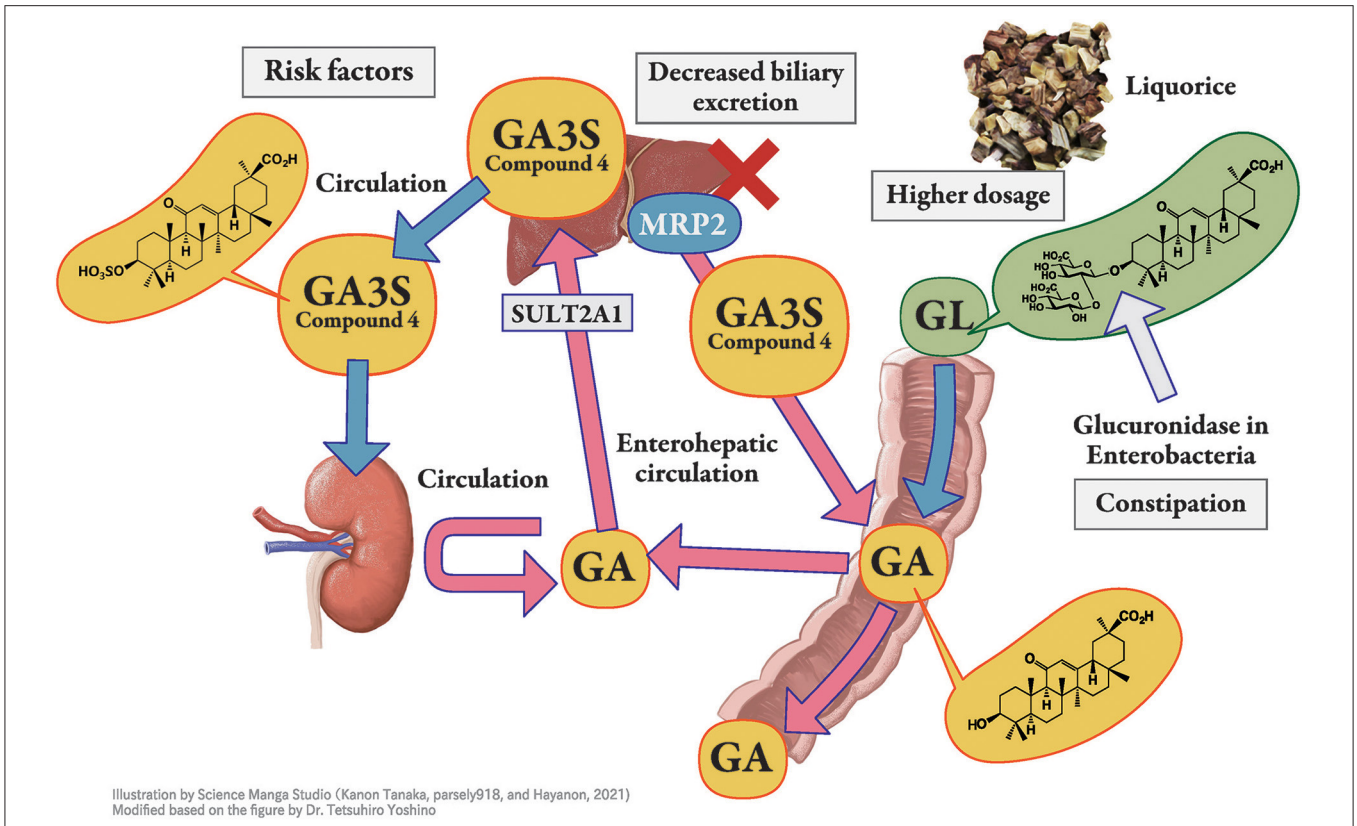

FIGURE 2 | Pharmacokinetics of glycyrrhizin-metabolites. Licorice, the root or stolon of Glycyrrhiza glabra or G. ularensis, contains glycyrrhizin (GL), a glycoside of glycyrrhetinic acid (GA) with two molecules of glucuronic acid. When GL is administered orally, it is hydrolyzed to GA by the intestinal bacteria and then absorbed into blood circulation. GA in blood circulation is not excreted into the urine but is transferred into the liver, sulfated to GA-3-*O*-sulphate (GA3S) by sulfotransferase (SULT) 2A1, and excreted into bile via multidrug resistance-associated protein (MRP) 2. In humans, GA3S is detected at a higher concentration than GA. In the intestine, GA3S is hydrolyzed to GA by the intestinal bacteria and is partially absorbed from the intestine into blood circulation to exhibits enterohepatic circulation, whereas the unabsorbed portion of GA3S is excreted in the feces. Orally taken GL is effectively hydrolyzed to GA by intestinal bacteria in constipated patients, which enhances its bioavailability. Hyper direct-bilirubin could thus be a surrogate marker of multidrug resistance-associated protein 2 dysfunction.

extract prepared with 1.5 g licorice (43, 49). Loop diuretics block Na-K-2Cl cotransporters, and thiazides block Na-Cl transporters in the distal nephron. Although these diuretics can prevent peripheral edema and hypertension, they increase intraductal flow into the collecting duct and stimulate sodium reabsorption (58), which increases potassium excretion and induces hypokalemia in patients taking licorice-containing products. Systemic glucocorticoid use can also aggravate the inhibition of 11βHSD2 that is similar to Cushing syndrome.

Conversely, potassium-sparing medications such as aldosterone blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers prevent licorice-induced hypokalemia. Concomitant use of these medications thus hinders the early detection of licorice-induced PsA.

Dementia

Dementia also makes early detection of PsA difficult (43). Therefore, medical specialists, including pharmacists and nurses, should be aware of hypertension or peripheral edema in the patient, even though these physical signs are not very sensitive or specific for licorice-induced PsA.

CONCLUSIONS

We summarized the current understanding regarding the pathophysiology of licorice-induced PsA and listed several factors that affect the pharmacokinetics of GL metabolites, including the daily dosage, dosing period, constipation, hypoalbuminemia, and hyperdirect-bilirubin. Further, older age and several concomitant medications are risk factors for licorice-induced pseudoaldosteronism. Importantly, a deep understanding of the crude drugs in Kampo preparations and the pathophysiology of licorice-induced PsA is necessary for the prevention and early diagnosis of PsA. Clinicians should be aware of PsA and balance the merit and demerit of using licorice or GL for therapy.

AUTHOR CONTRIBUTIONS

TY, SS, MH, and TM wrote the draft of the manuscript. MM and KW designed the study and revised the manuscript. All authors read and approved the final manuscript.

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Daijokito Administration in Critically Ill Patients Increasing the Stool Volume: A Retrospective Observational Study

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Introduction: *Daijokito*, a traditional Japanese herbal medicine (Kampo), has been used to treat abdominal distention of the middle yang stage pattern. The use of *Daijokito* has not been thoroughly investigated in critical care. To investigate a new Kampo approach to defecation control in critically ill patients, our study aimed to assess the effects of *Daijokito* on fecal management.

Methods: We analyzed 30 consecutive patients treated with *Daijokito* in the intensive care unit (ICU) between March 2017 and February 2021. The eligibility criteria were patients who were newly prescribed *Daijokito* in the ICU during the study period. Exclusion criteria were patients who were started on other laxatives within one day of beginning *Daijokito*. The study's primary outcome was defecation volume three days before and three days after starting *Daijokito*. We recorded the most dominant stool quality within three days after the start of *Daijokito*.

Results: Twenty-one patients were included in the analysis. The median age was 69.0 years, and the median sequential organ failure assessment score on admission to the ICU was 6.0. Major diseases included trauma, pancreatitis, and burns. Administration of *Daijokito* resulted in defecation in 17 of twenty-one patients (81.0%). Comparison of defecation volume between 3 days before *Daijokito* administration and three days, including the day of *Daijokito* administration, showed that defecation volume increased significantly after *Daijokito* administration, with a median of 0 to 360 g ($p < 0.001$). At the three-day follow-up, six of 17 (35.3%) patients defecated on the day of *Daijokito* administration, and nine (52.9%) defecated on the day after administration. One patient was judged to have excessive defecation, and *Daijokito* administration was discontinued. Stool quality was normal in one (5.9%) of the 17 patients, soft-formed in two (11.8%), loose-unformed in 11 (64.7%), and liquid in three (17.6%).

Discussion: *Daijokito* administration in critically ill patients caused defecation in 81% of the patients and significantly increased stool volume. The novelty of this study is that it sheds light on the Kampo treatment of defecation control in critically ill patients. In addition to the present report, further studies are warranted to quantify the therapeutic efficacy and safety of *Daijokito*.

Keywords: Kampo, critical care, intensive care units, constipation, laxatives

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INTRODUCTION

Most doctors in Japan are reported to use Kampo, traditional Japanese herbal medicine (1). The Kampo medicine *Daijokito* (DJT) is composed of the following herbs: Magnolia bark, immature orange, rhubarb rhizome, and anhydrous mirabilitum (**Figure 1**). In Kampo medicine, magnolia bark and immature orange regulate and normalize the flow of qi. Rhubarb rhizome and anhydrous mirabilitum have purgative properties and remove heat toxins [Traditionalmedicinemodule1 : TM1] in the intestinal tract. Therefore, DJT has been used to treat abdominal distention, constipation, wheezing, and psychological symptoms in the middle yang stage pattern. Chapter 208 in Shanghanlun says that "When in middle yang stage pattern, the pulse is slow, thought there is sweating, but aversion to cold is absent, there will be generalized heaviness, shortness of breath, abdominal fullness, panting, and tidal heat effusion, which means the exterior [TM1] is about to resolve and one can attack the interior[TM1]. Sweat streaming from the extremities indicates that the stool is already hard, the DJT governs." DJT is used in clinical settings to treat significant constipation, hypertension, neurosis, and food poisoning. It has also been reported as a treatment for acute pancreatitis, paralytic ileus, and tetanus in intensive care medicine (2–4).

Among Kampo medicines for gastrointestinal motility, Daikenchuto is the most investigated. Some randomized controlled trials have shown that Daikenchuto significantly enhanced ascending colon emptying compared to a placebo (5); Daikenchuto reduced postoperative ileus surgery and postoperative ileus recurrence (6). In the Japanese guidelines for nutrition support therapy in critically ill patients, Daikenchuto is expected to be a potentially effective treatment for improving gastrointestinal motility (7). However, DJT has not been well investigated. DJT contains rhubarba with stimulant laxative action (8); thus, compared with Daikenchuto, which does not include rhubarba, DJT should be a more potent agent to induce intestinal motility.

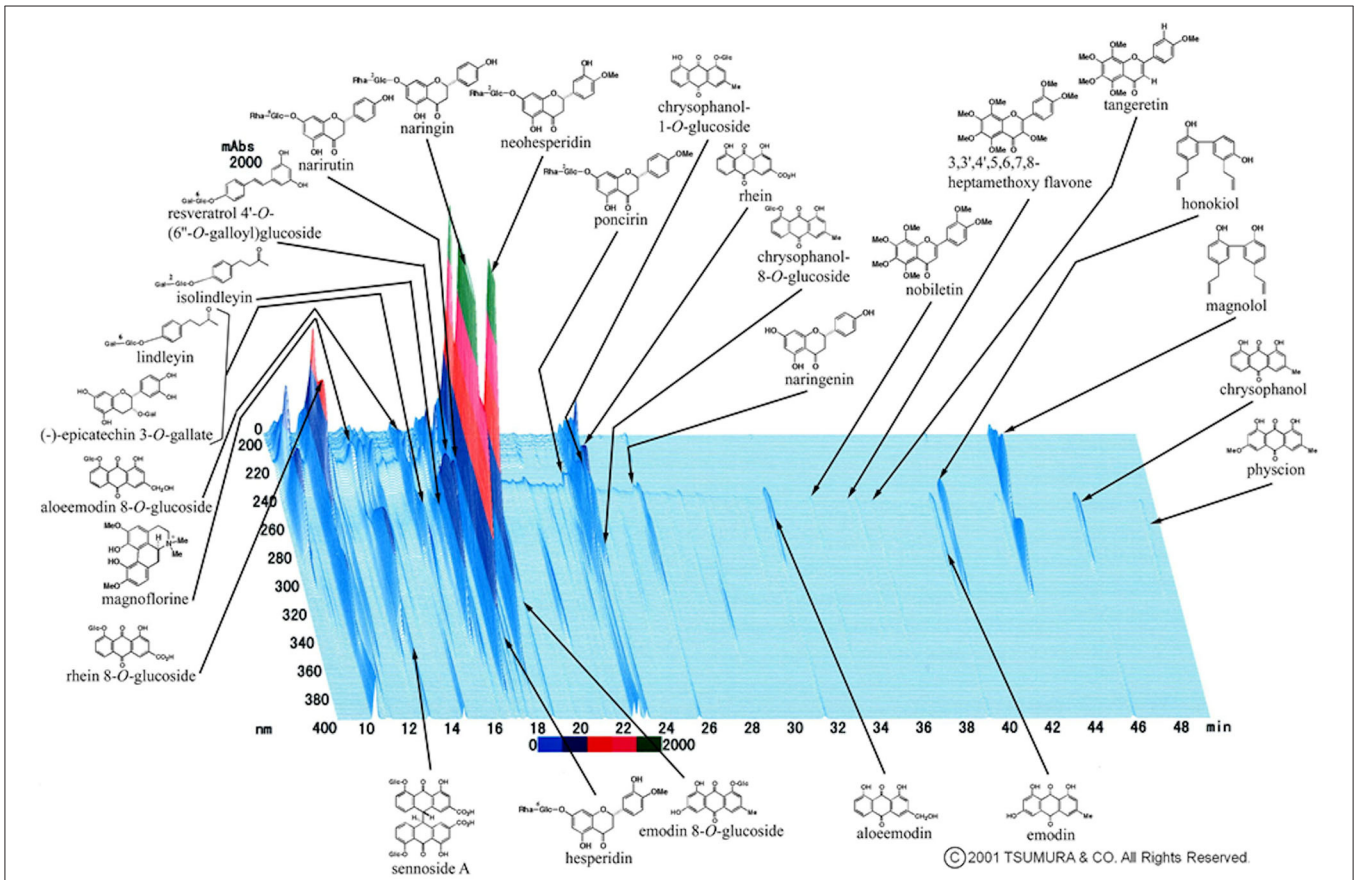

FIGURE 1 | Three-dimensional high-performance liquid chromatography profile of *Daijokito* (provided by Tsumura & Co.). A three-dimensional high-performance liquid chromatography (HPLC) chart of the methanol solution of DJT. DJT was prepared with 20 mL of methanol under ultrasonication for 30 min. The solution was filtered and subjected to HPLC analysis. HPLC equipment was controlled with an HPLC pump (LC-10AD; Shimazu, Kyoto, Japan) using a TSK-GEL ODS-80TS column (4.6ϕ × 250 nm), and elution was performed using solvents (A) 0.05 M ammonium acetate (AcONH4; pH 3.6) and (B) acetonitrile (CH_3CN). A linear gradient of 100% A and 0% B for 60 min to 0% A and 100% B was used. The flow rate was controlled with LC-10AD at 1.0 mL/min. The eluate from the column was monitored, and the 3D data were processed using a diode array detector (SPD-M10A; Shimadzu, Kyoto, Japan).

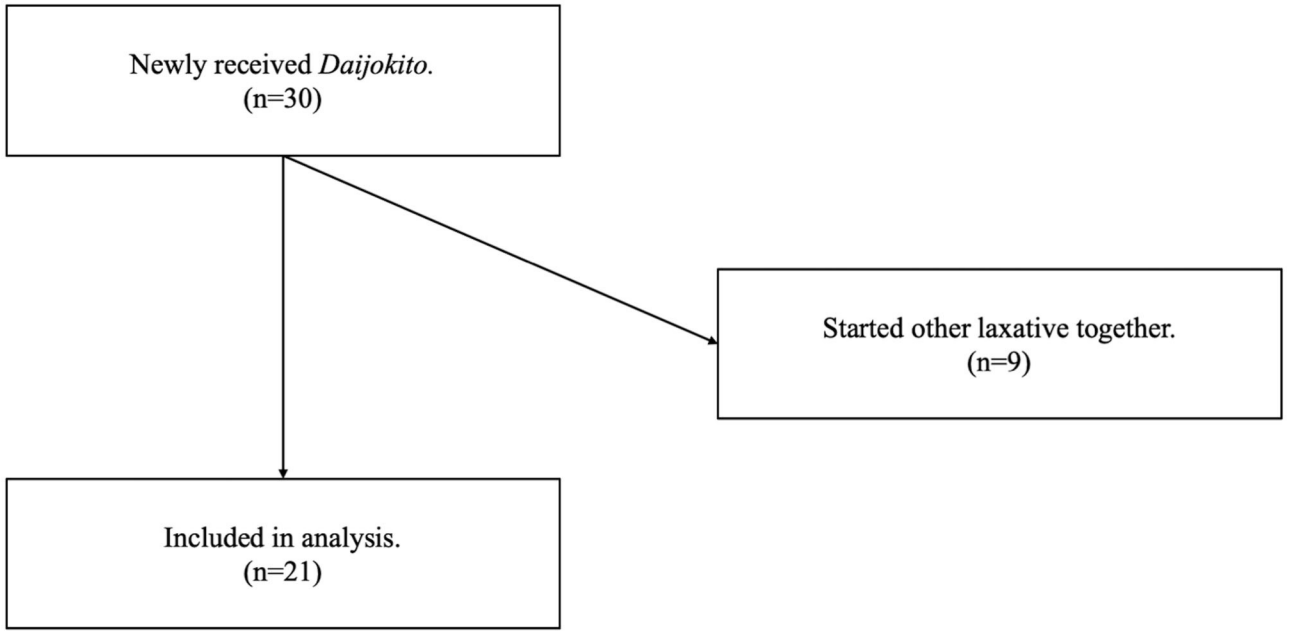

FIGURE 2 | Flow of patients in a study of the effect of *Daijokito* in critical care unit.

Constipation is frequently reported in 50–83% of critically ill patients (9, 10). Patients with more severe illness are more likely to have constipation (11). Constipation is reported to be associated with adverse events such as ventilator weaning failure (9) and delirium (12). Thus, defecation control in critically ill patients has important implications.

To investigate a new Kampo approach to defecation control in critically ill patients, our study aimed to assess the effect of DJT on fecal management in critically ill patients.

MATERIALS AND METHODS

Study Design and Participants

We analyzed 30 consecutive patients treated with DJT in the intensive care unit (ICU) of Akita University Hospital between March 2017 and February 2021. Akita University Hospital is a tertiary care hospital in a rural area and has 16 ICU beds. This study was a retrospective observational cohort study and conformed to the principles of the Declaration of Helsinki. The ethics committee of Akita University Hospital approved the study protocol. The need for informed consent was admitted waiving because of the observational nature of the study and the requirement for no treatments beyond the daily clinical practice. The eligibility criteria were patients who were newly prescribed DJT in the ICU during the study period. The exclusion criteria were patients who were started on other laxatives within one day of beginning DJT.

DJT

The decision of who to administer DJT was left to the clinician. Clinicians often initiate DJT based on the following factors: small volume of defecation, prolonged absence of stool, abdominal physiological findings, and abdominal radiographic findings. DJT was administered three times daily, one pack at a time via nasogastric tube. The DJT used in this study was produced by Tsumura & Co. (Tokyo, Japan) (8).

Outcome

The study's primary outcome was defecation volume three days before and three days after starting DJT. In addition, the number of constipated days, number of days between the start of DJT and defecation, stool quality, and survival at discharge were recorded. If a new laxative was added before defecation, we judged that DJT was ineffective and recorded the stool volume as zero. Stool quality was classified into four categories: normal, soft-formed, loose-unformed, and liquid. We recorded the most dominant stool quality within three days after the start of DJT.

Statistical Methods

To determine if there was a difference in each variable between the pre - and post-DJT administration, we used the Wilcoxon signed-rank test to assess the significance level at 5%. There were no missing data. Statistical analyses were conducted using Stata® software (version 16.1; StataCorp, College Station, Texas, USA). Significance was defined as a two-sided p-value of < 0.05.

RESULTS

Of the 30 patients newly received DJT in the ICU, nine were met the exclusion criteria because they started other laxative treatments at the same time. Therefore, we analyzed twenty-one patients (**Figure 2**). The median age was 69.0 years, and the proportion of male patients was 52.4%. The median SOFA score (13) on admission to the ICU was 6.0, and the median

TABLE 1 | Characteristics of the patients included in the current study.

	Variables	
Age (years old)	69 (57–80)	
Male	11/21 (52.4%)	
SOFA score	6 (4-8)	
Constipation day (days)	2 (1-3)	
Diagnosis	Trauma	4 (19.0%)
	Pancreatitis	3 (14.3%)
	Burn	3 (14.3%)
	Sepsis	2 (9.5%)
	Tetanus	2 (9.5%)
	Intoxication	2 (9.5%)
	Heatstroke	1 (4.8%)
	Carbon dioxide narcosis	1 (4.8%)
	Acute heart failure	1 (4.8%)
	Neuroleptic Malignant Syndrome	1 (4.8%)
	Post-cardiac arrest syndrome	1 (4.8%)
Opioid use	18/21 (85.7%)	
Enteral nutrition use	20/21 (95.2%)	

Data are expressed as medians (interquartile ranges) for continuous variables and numbers (%) for categorical variables. SOFA, Sequential Organ Failure Assessment.

duration of no defecation before DJT administration was two days. The patients' major diseases included trauma, pancreatitis, burn, sepsis, tetanus, and intoxication. The patient characteristics are shown in **Table 1**. No patients underwent abdominal surgery.

Of the twenty-one patients, we attempted to control defecation in 13 patients (61.9%) with other laxatives before DJT, five patients (23.8%) received one laxative, five patients (23.8%) received two laxatives, two patients (9.5%) received three laxatives, and one patient (4.8%) received four laxatives. As for the type of laxative, oral sodium picosulfate solution was used for six (28.6%), oral Daikenchuto, a herbal medicine, for six (28.6%), bisacodyl suppository for five (23.8%), oral naldemedine tosilate for three (14.3%), oral sennoside for two (9.5%), oral magnesium citrate solution, oral magnesium oxide, and glycerin enema for one (4.8%). As these drugs were considered inadequate, DJT was administered.

Administration of DJT resulted in defecation in 17 of 21 patients (81.0%). Comparison of defecation volume between 3 days before DJT administration (Pre-DJT group) and three days, including the day of DJT administration (Post-DJT group) showed that defecation volume increased significantly after DJT administration with a median of 0 g (interquartile range [IQR], 0–100) in the Pre-DJT group and 360 g (IQR, 148–560) in the Post-DJT group ($p < 0.001$, **Figure 3**).

At the three-day follow-up, including the day of DJT administration, six of 17 (35.3%) patients defecated on the day of DJT administration, nine (52.9%) defecated on the day after administration, and two (11.8%) defecated two days after administration. One patient was judged to have excessive defecation, and DJT administration was discontinued on the second day. Stool quality was normal in one (5.9%) of the 17 patients, soft-formed in two (11.8%), loose-unformed in 11 (64.7%), and liquid in three (17.6%). There were four deaths out of twenty-one patients at discharge, with a mortality rate of 19.1%.

DISCUSSION

DJT administration in critically ill patients causes defecation in 81% of patients and significantly increases stool volume. Therefore, DJT is expected to improve lower gastrointestinal motility in intensive care units. Most of the defecation occurred on the day of administration or the next day, and loose-unformed was the most common stool quality. This study is novel as it is one of the first to shed light on the Kampo treatment of defecation control in critically ill patients. To the best of our knowledge, this is the first study to focus on DJT.

First, at the 3-day follow-up after DJT administration, 81% of patients showed an increase in stool volume, and more than half of these patients had bowel movement the day after DJT administration. The median stool volume increased from 0 g per 3 days to 360 g every 3 days. Few previous studies have evaluated the effect of individual laxatives on defecation volume in critically ill patients. For instance, in patients with constipation with multiple organ failure, lactulose promoted defecation in 69% of patients (median time to defecation was 36 h), and polyethylene glycol stimulated defecation in 74% of patients (median time to defecation was 44 h) (14). DJT may be competitive with these typical laxatives. Moreover, about 60% of our patients were administered DJT because other laxatives were ineffective. We propose DJT as a treatment option for refractory lower gastrointestinal motility failure in critically ill patients.

Second, loosely unformed stool was the most common stool quality, and about 82.4% of the patients presented with diarrheal stools. Almost all of our patients received parenteral nutrition. Considering that diarrhea occurred in 18% of patients receiving enteral feeding (15), the incidence of diarrhea in patients receiving DJT was high. However, only one patient (5.9%) discontinued DJT administration because of clinically determined excessive defecation, and DJT was unlikely to cause diarrhea with adverse effects, such as water and electrolyte imbalance.

The basic strategy for treating constipation is stool softening by regulating the intestinal tract's water content and enhancing bowel motility by stimulating the intestinal mucosa. The anhydrous mirabilitum in DJT has a stool softening effect, and rhubarb has a hypermotility effect, and they are traditionally utilized couplings (16). Rhubarb is reported to enhance the effect of anhydrous mirabilitum in the intestinal tract and accelerate the onset of purgative action (16). Magnolia bark has psychotropic effects (17), and immature orange has anti-inflammatory effects. Such synergistic effects and multifunctionality are the strong points of Kampo medicine. While the negative effects of polypharmacy may occur to cover various effects with western medicines, the combination of crude drugs in Kampo medicine has been sophisticated throughout history. Considering that many patients in the ICU have systemic inflammatory syndromes and that constipation is associated with delirium in critically ill patients (12), the various effects of DJT may be appropriate in the ICU.

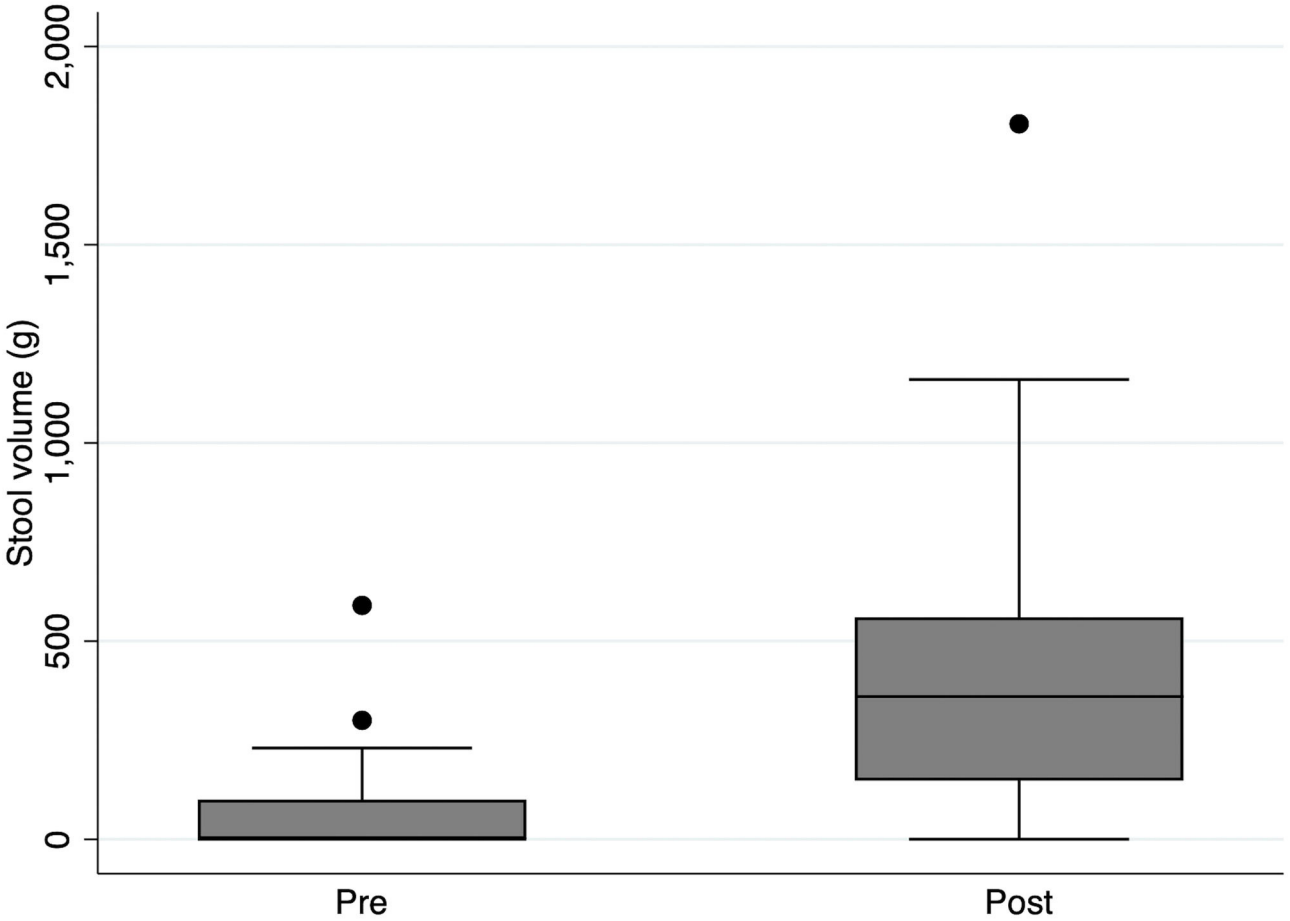

FIGURE 3 | Comparison of stool volume pre and post intervention with *Daijokito*. Comparison of stool volume between 3 days before DJT administration (Pre-DJT group) and three days after, including the day of DJT administration (Post-DJT group) showed that stool volume increased significantly after DJT administration with a median of 0 g (interquartile range [IQR], 0–100) in the Pre-DJT group and 360 g (IQR, 148–560) in the Post-DJT group ($p < 0.001$).

Our study has two limitations. First, the present study was a pre- and post-observational study and did not exclude various biases and confounding factors. And the design of this study did not exclude the possibility that defecation occurred in the natural course without the use of DJT, and that the phenomenon of regression to the mean was observed. Second,the introduction/termination of DJT was left up to the clinician, which was potentially subject to bias. In particular, DJT tends to be initiated in constipation refractory to multiple laxatives, and the major limitation is the inability to distinguish the effects of various laxatives from those of DJT. In addition to the present report, further studies are warranted to quantify the therapeutic efficacy and safety of DJT.

In conclusion, the administration of DJT caused defecation in critically ill patients and significantly increased the stool volume. Although loose unformed stools were the most common stool quality, only one patient had to discontinue DJT administration. Therefore, DJT may improve lower gastrointestinal motility in patients the intensive care unit; however, further high-quality studies are needed to establish the reliability of DJT.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Akita University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KS designed the study and wrote the initial draft of the manuscript. HN contributed to the conception of the study and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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"Kambakutaisoto" and Emotional Instability Associated With Premenstrual Syndrome

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Many women suffer from premenstrual syndrome (PMS), which can be considered a modern illness in this busy society; mental symptoms, such as irritability, often affect the surroundings and result in loss of self-confidence. The symptoms of PMS are diverse, and it is often difficult to treat psychiatric and social symptoms with low-dose estrogen progestin combination drug (LEP) alone. Selective serotonin reuptake inhibitors (SSRIs) are also effective; however, many are unable to take them owing to their side effects. "Kambakutaisoto" is a Kampo medicine consisting of "jujube," "licorice," and "wheat," which is often described as "food"; however, it is highly effective in treating emotional instability attributed to PMS in sensitive young women. There are many reports on the effects of kambakutaisoto; the molecular nutritional findings of kambakutaisoto, which has dramatic effects despite its mild composition of crude drugs, have also been reported, suggesting an association with premenstrual exacerbation of functional hypoglycemia. A narrative review of its clinical effects on PMS and the results of molecular nutrition studies was performed.

Keywords: premenstrual syndrome (PMS), Japanese Kampo medicine, kambakutaisoto, functional hypoglycemia, tryptophan

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INTRODUCTION

Many women today suffer from premenstrual syndrome (PMS) (1). PMS can be considered a modern illness associated with a busy lifestyle; mental symptoms such as irritability often affect one's surroundings and results in a loss of self-confidence (2).

The cause of PMS has not yet been elucidated in detail, although there is a theory that serotonergic neurons are highly sensitive to progesterone (3). The symptoms of PMS are diverse, and it is often difficult to treat psychiatric and social symptoms with low-dose estrogen progestin combination drug (LEP) alone (4). Serotonin reuptake inhibitors (SSRIs) are effective; however, many patients are unable to take them owing to their side effects (3). Kampo medicine could potentially be effective in the treatment of PMS if Kampo medicine is selected based on a pattern called "sho" that is specific to Kampo medicine. "Kambakutaisoto" is a Kampo medicine consisting of jujube, licorice, and wheat, which is often described as food; this medicine is highly effective in treating emotional instability attributed to PMS in sensitive young women (5–15).

Why does kambakutaisoto have a dramatic sedative effect even though it is a mild formulation of crude drugs that could be considered to be food? One answer is that, from a molecular nutritional point of view, kambakutaisoto stabilizes blood sugar and contains components involved in the production of serotonin and gamma amino butyric acid (GABA) (10, 16). Is it related to the

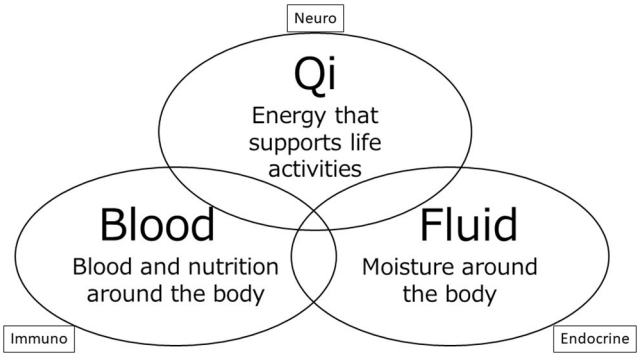

FIGURE 1 | Theory of Qi, Blood, Fluid. The three elements showing in this figure are thought to maintain the living body within the Kampo medicine framework. Three elements go around the mind and body, and make them work. Symptoms are caused by impaired circulation and quantity of three elements.

recently discussed mechanism of functional hypoglycemia? During the luteal phase, functional hypoglycemia could be exacerbated and manifested as a symptom of PMS (17, 18). Kambakutaisoto could be effective in treating hypoglycemia as an underlying cause. Hence, we have reviewed the effects of kambakutaisoto on PMS, the molecular nutritional content of kambakutaisoto, and functional hypoglycemia.

DIAGNOSIS AND TREATMENT IN KAMPO MEDICINE

In Kampo medicine, which is a traditional Japanese medicine, various crude drugs work in conjunction, presenting the effects of mutual relationships among crude drugs. The crude drugs are mainly natural and gentle, such as grassroots bark.

Kampo medicine has gradually begun to reemerge and now occupies a considerable position in the field of medical practice in Japan. We introduce one of the theories of choosing Kampo medicine in **Figure 1**.

In Kampo medicine, qi is considered a life energy source that is sourced from food and air. The concept of qi plays a primary role in the assessment of clinical condition in Kampo medicine, where physical disorders indicate abnormal quantities or locations of qi. In the body, part of qi becomes a liquid in order to build and maintain life. Among the liquids, red liquid is blood, and the remaining colorless liquids are fluid. The three entities of qi, blood, and fluid bear a close relation to the neuro-immuno-endocrine triangle familiar to Western medicine. This key concept is the basis for assessing the clinical conditions in Kampo medicine (19).

PMS AND KAMPO MEDICINE

According to the Kampo medical interpretation, the luteal phase is the time when qi and blood flow downward (assuming pregnancy). If there is qi depression or static blood in the base, various symptoms are likely to occur as PMS. Moreover, mental symptoms, such as irritability, often affect the surroundings and result in loss of self-confidence (2). Many Kampo medicines have psychotropic effects. Kamishoyosan is a formulation against static blood and qi depression, and there are many reports on its effective in PMS (20–23). Shiota and Hata examined 38 cases of PMS (5). For those who felt betrayed by their expectations of others and turned their anger toward others, kamishoyosan was an effective treatment. Yokukansankachimpihange was effective for those who felt betrayed by their expectations for themselves and expressed anger toward themselves. Kambakutaisoto was effective for those who were delicate, emotionally unstable, and could not see themselves objectively (5).

PMS AND "KAMBAKUTAISOTO"

Since 2005, there have been reports of the use of kambakutaisoto for PMS and premenstrual dysphoric disorder (PMDD) (5). Nakai et al. reported that kambakutaisoto was dramatically effective in 14 cases of PMS (13–15). In many of these reported cases, 2.5–5 g of kambakutaisoto was used as a single dose as needed. In effective cases, it takes <1 h from the time of taking the drug until the effect is realized, and in many cases, the effect is seen within 30 min. **Supplementary Table 1** shows 32 cases in which the reported kambakutaisoto formulation was effective. A common feature of the 32 cases was uncontrollable premenstrual emotional instability. According to a review by Nakai et al. (15), which summarizes 14 cases in detail, many symptoms, such as yawning (86%, 12/14), edema (79%, 11/14), insomnia or drowsiness (64%: 9/14), and constipation (64%, 9/14) were reported. Impulsive symptoms, such as overeating (50%, 7/14), were also observed. In other studies, overeating, insomnia, constipation, and fatigue were common. Edema and constipation are findings of fluid retention and static blood, and are common symptoms in premenstrual women; hence, it cannot be assumed to be a characteristic symptom of women presenting the effect of kambakutaisoto. Regarding the findings of the Kampo assessment for 14 cases with abdominal examination, fluids retention in stomach, palpitation in the supra-umbilical region, and para-umbilical tenderness and resistance, were observed in nearly 80% of the cases. There were findings of fluid retention and static blood. Fullness and discomfort in the chest and hypochondrium and abdominal muscle tension, which are thought to be reflective of excessive stress, were also observed in approximately half the cases. In other words, if fluid retention or static blood is found in the abdominal examination, the corresponding Kampo medicine should be taken regularly. If one has impulsive symptoms, such as emotional instability or overeating, effects of kambakutaisoto can be treated with a single dose.

KAMBAKUTAISOTO: OUTSTANDING CASES AND FUNCTIONAL HYPOGLYCEMIA

Kambakutaisoto is a Kampo medicine consisting of jujube, licorice, and wheat. The details are listed in STORK (http://

mpdb.nibiohn.go.jp/kconsort/kconsort.html) (24). The original text describing this is "Jinguiyaolue": Women's Chronic Miscellaneous Diseases," which was written 1,800 years ago. According to Classics of traditional medicine, it is effective against hysterical attacks, sadness, crying, and yawning symptoms in women who appear as if they are possessed (25). The insurance coverage for Japanese Kampo medicine manufacturers includes crying at night, neurosis, and insomnia. In addition, it is widely used for urgent, unexplained frustration and excitement as well as convulsions.

We considered the characteristics of crude drugs that make up kambakutaisoto. Licorice has tension-relieving, sedative, analgesic, and stomach-healing effects, and "Yakucho" is said to cure urgency (26). Jujube has the effect of strengthening the digestive tract, stabilizing the mind, and relieving tension, and "Yakucho" is said to stop cramping (27). Although there is little mention of wheat in the literature, the "Bencao Gangmu (Materia Medica)" states that it nourishes the mind and is thought to have the effect of supplementing energy and stabilizing the mind.

All three crude drugs have mild conditions; however, in clinical practice, they show a sharp effect in a short amount of time. So far, it has been argued that the underlying mechanisms include a blood glucose-retaining effect, effects of tryptophan (involved in the production of serotonin) in the oral cavity, and endorphins secretion (10, 15). However, this has not been verified.

The concept of functional hypoglycemia has been advocated since the 1980s (28, 29). This concept was introduced to Japan by Osawa (30) in the 1990s and, along with Kashiwazaki, is actively implemented in treating patients (31, 32). The pathology is thought to be associated with eating a diet with rapidly fluctuating blood glucose levels (a diet with a high glycemic index: a GI diet), which overreacts or disrupts glycemic control. "Hypoglycemia" is generally associated with diabetes treatment. In addition, insulinoma often occurs in "fasting hypoglycemia" and "postprandial hypoglycemia (reactive hypoglycemia)" often occurs in the so-called dumping syndrome following gastric surgery and in the early stages of type 2 diabetes. However, hypoglycemia could occur even in people without such a background; hypoglycemia has been confirmed by oral precision glucose tolerance test (OGTT), and many cases have been reported in which improvement of dietary habits, such as sugar restriction, was effective (33–35).

When the blood sugar level rises sharply owing to a high GI diet, a large amount of insulin is secreted to regulate it in an attempt to lower the blood sugar level. When trying to raise the blood sugar levels that have dropped too low, hormones such as adrenaline, noradrenaline, and cortisol are also rapidly secreted. In other words, after an uplifting mood due to hyperglycemia, a rapid decrease in blood glucose levels occurs owing to excessive insulin secretion. Following this, drowsiness, yawning, poor concentration, and tiredness appear. Next, anger, frustration, and aggressive behavior owing to the adrenaline secreted to raise blood sugar appears. Similarly, anxiety, sadness, and alexithymia owing to noradrenaline secretion are observed. This causes an urge to eat sweets, and the cycle is repeated again (16) (**Figure 2**). It is very surprising that these symptoms are consistent with the rules of kambakutaisoto and the symptoms that apply to prominent cases.

Several studies have been conducted on the relationship between the female menstrual cycle and blood glucose. During the follicular phase, blood glucose tends to be low due to increased insulin sensitivity and decreased insulin resistance owing to the effects of estrogen. It is said that during the luteal phase, insulin sensitivity decreases and insulin resistance increases owing to the effects of progesterone, resulting in an increase in blood glucose (18).

It has also been reported that 70% of women with type 1 diabetes had elevated blood glucose before menstruation and half of them decreased on the first day of menstruation (36).

Among PMS, those with strong mental symptoms are called Premenstrual Dysphoric Disorder (PMDD), and the diagnostic criteria include the item of "overeating." Even women who cannot be diagnosed with PMDD, PMS often feel increased appetite. If blood glucose tends to be high during the luteal phase, why is appetite increased?

It has been reported that during the luteal phase, progesterone acts as a mechanism for mobilizing glucose and accumulating fat, promoting hunger and increased appetite (37). Leptin levels have been reported to be associated with overeating in women with normal weight PMDD (38). In addition, since PMS symptoms are more likely to occur in women who are highly sensitive to progesterone in serotonergic neurons, it is possible that a decrease in serotonin increases appetite. There is also a report that after absorption of the sweet solution, serotonin in the brain increased and the mood improved (39). Appetite is also increased as a simple method of coping with stress (40). When overeating occurs in the luteal phase in this way, reactive hypoglycemia is likely to occur because of impaired glucose tolerance following after decreased insulin sensitivity, compared to the follicular phase. This is similar to reactive hypoglycemia in the early stages of type 2 diabetes. It is conceivable that those who have "functional hypoglycemia" as a base, the condition may worsen during the luteal phase; as a result, symptoms may become apparent.

There are studies examining blood glucose in patients with PMS and the control group (41). According to the results, the blood glucose level in the luteal phase was significantly lower than that in the control group. However, since this study is a one-point test of blood sampling results, it may not be an accurate representation of the subject's postprandial hypoglycemic status.

MOLECULAR NUTRITIONAL CONSIDERATIONS

Is "Kambakutaisoto" really effective in improving the symptoms caused by functional hypoglycemia? To support this, we would like to introduce the molecular nutritional consideration of kambakutaisoto, as clarified by Shime et al. (16).

These researchers asked the Japan Food Research Laboratories to measure 55 items, such as proteins, lipids, electrolytes, carbohydrates, sugar, dietary fiber, energy, vitamins, and amino acids (**Supplementary Table 2**) (100 g), sugar (84.8 g; fructose,

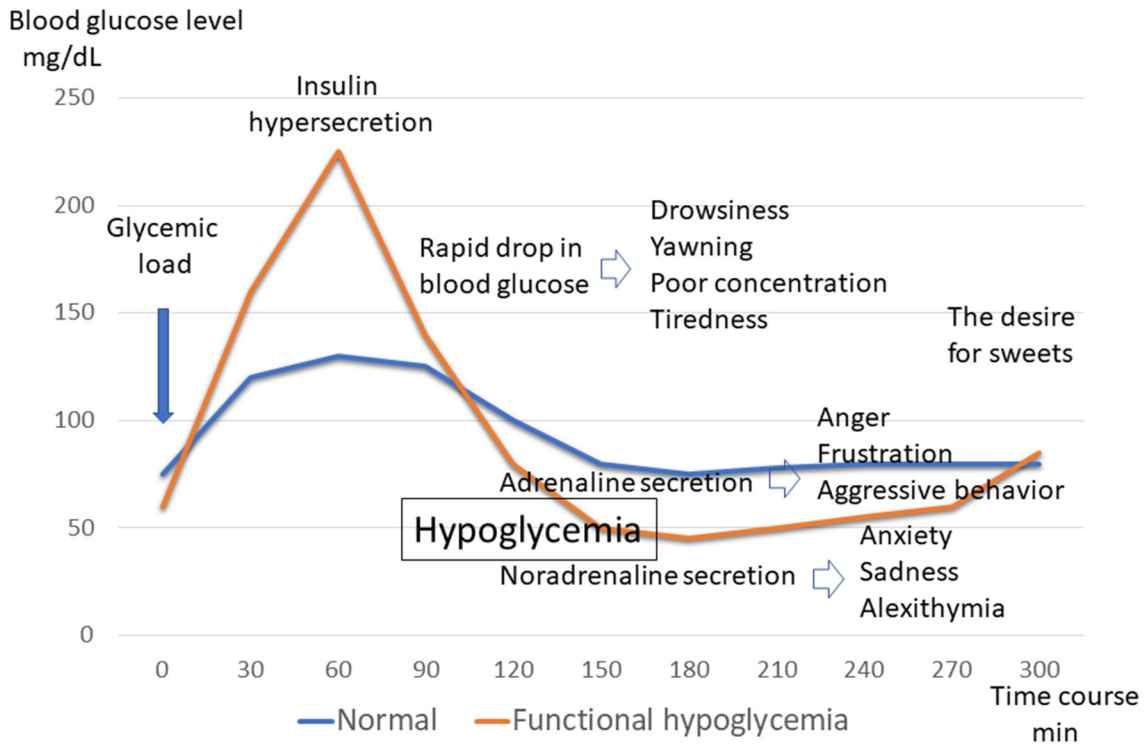

FIGURE 2 | Blood glucose levels and symptoms of functional hypoglycemia. Glycemic fluctuations in normal and functional hypoglycemic patterns following glucose loading. Changes in symptoms associated with glycemic fluctuations in functional hypoglycemia.

8.85 g; glucose, 7.87 g; sucrose, 2.51 g; maltose, 0.21 g; lactose, 51.5 g), dietary fiber (3.8 g), protein (3.7 g), lipids (1.7 g), iron, calcium, potassium, magnesium, copper, zinc, manganese, vitamins B1, B2, B6, B12, and K, folic acid, pantothenic acid, biotin, and niacin, 18 amino acids, tryptophan (25 mg), and glutamic acid (342 mg) were observed. Based on these results, the following is suggested.

1. Monosaccharides and disaccharides are confirmed as sugars, and dietary fiber is also included to stabilize blood glucose disorders attributed to functional hypoglycemia.
2. This mixture contains tryptophan, minerals, and vitamins for conversion to serotonin and melatonin. It also contains glutamic acid, which promotes GABA production.
3. Various nutrients related to these systems can be ingested at the same time and act as a complex system.

The basic treatment for functional hypoglycemia is improvement of eating habits; however, it is not cured in a short period of time; it often takes several years or more to improve symptoms. If the symptoms worsen, they can be alleviated by taking a small amount of low GI diet every few hours, though we consider kambakutaisoto effective during such times.

Nakajima describes the relationship between kambakutaisoto and intestinal flora as follows (42): wheat contains all the ingredients necessary for tryptophan metabolism by the intestinal flora. However, its metabolism is directed toward kynurenine production, which causes mental disorders, rather than serotonin production under chronic inflammation and stress. Licorice exerts corticosteroid action through the intestinal flora. Due to its anti-inflammatory effect, tryptophan metabolism lowers kynurenine and leads to serotonin production.

Indeed, the effects of Kampo medicine are complex, and the relationship between the nutritional aspects of crude drugs and the intestinal flora is also interesting.

KAMPO MEDICINE: A MULTI-COMPONENT SYSTEM

Kampo medicine is composed of multiple botanical ingredients, including many pharmacologically active substances. For quality control, it is important to make efforts to stabilize the quality of raw materials at the crude drug level. The 3D-HPLC (High Performance Liquid Chromatography) fingerprint evaluation method is useful for quality control of crude drugs and final products (43). This is one of the methods proposed by the Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA) for the quality control of botanical drugs. The 3D-HPLC pattern of kambakutaisoto is shown in **Figure 3**.

Because Kampo medicine are multi-component, they have multiple targets. It is thought that these multi-ingredients of kambakutaisoto have a mild effect on blood glucose regulation and serotonin production from tryptophan, similar to an orchestra.

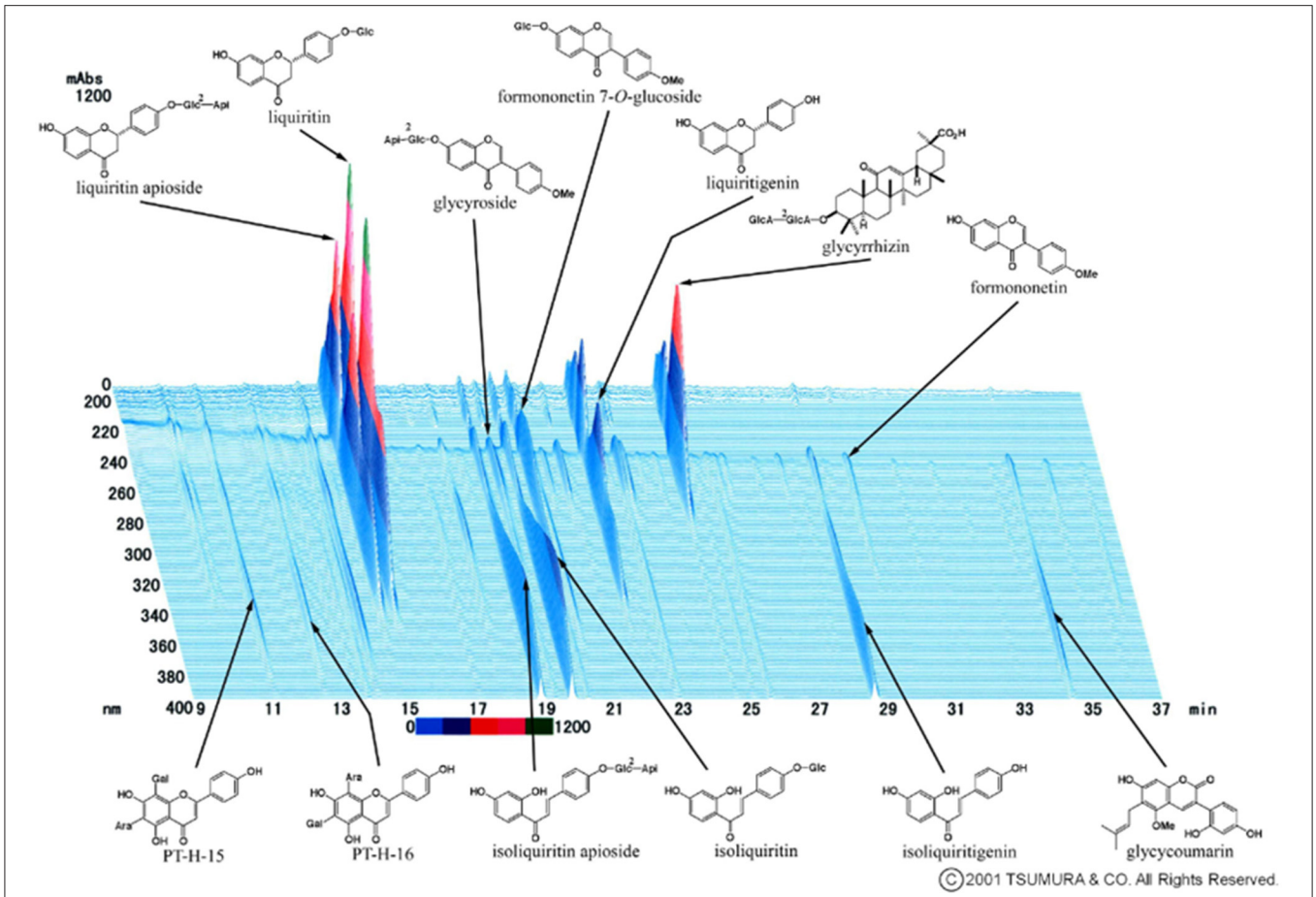

FIGURE 3 | 3D HPLC Pattern of Kambakutaisoto. 3D-HPLC (High Performance Liquid Chromatography) fingerprint evaluation of Kambakutaisoto. Using this method, the equivalence of herbal preparations consisting of botanical crude drugs is evaluated.

CONCLUSIONS

We have reviewed the clinical effects of the Japanese Kampo medicine kambakutaisoto on emotional instability associated with PMS, with "functional hypoglycemia" as a keyword, and added molecular nutritional considerations.

We hypothesized that some patients with PMS have symptoms of exacerbation of functional hypoglycemia during the luteal phase, and the characteristic symptoms are uncontrollable emotional instability and urgency. Kambakutaisoto, with molecular nutritional evidence, is very effective for such symptoms.

However, few studies have considered functional hypoglycemia in detail (33–35). There are fewer papers on PMS and glycemic fluctuations (41).

An accurate diagnosis of functional hypoglycemia requires a 5 h OGTT (31, 32).

To the best of our knowledge, there are no PMS research papers investigating hypoglycemia with a 5 h OGTT.

We believe that it is necessary to investigate blood glucose fluctuations in patients with PMS. Currently, there is an excellent glucose monitoring system that can be worn subcutaneously to measure diurnal variations in blood glucose levels in patients with diabetes. It would also be very meaningful to use it to investigate diurnal fluctuations in blood glucose levels in patients with PMS.

We wish to study the fluctuations in blood glucose among patients with PMS as well as blood glucose levels after taking kambakutaisoto. We hope that this review will lead to further research in this field.

AUTHOR'S NOTE

Kambakutaisoto extract granules for ethical use used in **Supplementary Tables 1, 2** and **Supplementary Figure 3** was made by Tsumura Co., ltd.

AUTHOR CONTRIBUTIONS

AS, CS, and KN contributed to conception and design of the study. AS and KN organized the database. CS made a molecular nutritional consideration. AS wrote the first draft of the manuscript. AS, CS, KN, and MK wrote sections of the manuscript. MK supervised the entire treatise. All authors

contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.760958/full#supplementary-material

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CASE REPORT
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Sho-Based Kampo Medicine Combined With Assisted Reproductive Technology Is Effective for Refractory Infertility and Early Recurrent Miscarriage: A Case Report

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Assisted reproductive technology (ART) is an effective treatment developed for infertile couples in the world. As a result, women suffering from infertility benefit from ART treatment. However, even when ART treatment is successfully performed, there are cases where conception is not achieved or maintained. Kampo medicine was originally developed in Japan, and *Sho* is the central part of Kampo concept. Although it is thought that Kampo medicine is useful for various women-specific symptoms in modern Japan, evidence is still lacking regarding the effectiveness of combination of *Sho*-based Kampo and Western medicine such as ART. In this article, we report a case of a patient with refractory infertility and early recurrent miscarriage (ERM) of unknown cause who successfully became pregnant with combination therapy of Kampo based on *Sho* and ART. The patient was a 34 year-old Japanese woman and had been treated with ART in a nearby clinic. In a 3 year period, she had undergone oocyte retrieval twice, frozen embryo transfer (FET) seven times and conceived twice. Since both conceptions ended in miscarriages and pregnancy could not be established thereafter, her clinic referred her to our hospital for Kampo treatment. As result of the diagnosis of her *Sho*-pattern, we chose Kampo medicine. Finally, she succeeded in conception 1.5 years after beginning treatment and was able to carry the fetus to term successfully. The current case showed that although our patient had been unable to give a birth after undergoing various western medical treatments for infertility, pregnancy was established and kept to term after addition of *Sho*-based Kampo treatment. Kampo medicine chosen by the *Sho*-patterns is useful for refractory infertility and ERM. It is important to note that examinations for evaluting the *Sho*-patterns are essential for selecting appropriate Kampo medicine. *Sho*-based Kampo leads to an increase in the effectiveness of ART treatment, and accumulation of evidence that clarifies *Sho*-pattern is required.

Keywords: Kampo, *Sho*, infertility, early recurrent miscarriage, assisted reproductive technology

INTRODUCTION

Women suffering from infertility due to ovarian insufficiency, tubal obstruction and even unknown causes have benefited from assisted reproductive technology (ART) treatment such as *in vitro* fertilization (IVF) and intracytoplasmic insemination (ICSI). The process of ART involves ovarian stimulation to produce multiple follicles, retrieval of the oocytes from the ovaries, oocyte fertilization and embryo incubation in the laboratory, and transfer of embryos into a women's uterus. Globally, the total number of ART cycles increased by almost 20% between 2011 and 2012, whereas pregnancy and delivery rates remained stable (1). In Japan, 454,893 treatment cycles were carried out in 2018, resulting in the birth of 56,979 neonates. The total number of both treatment cycles and neonates born in 2018 increased from 2017 (2). Although ART is now widely accepted as clinically effective for the treatment of many forms of infertility, some people remain unable to conceive due to poor reproductive function. In addition, the miscarriage rate per pregnancy established by ART in Japan is high, at 29.0% (2).

Kampo medicine originated around two thousand years ago in China. Herbal medicine grown in China was introduced to Japan in the Edo period (1603–1868), and from then it was uniquely developed as traditional Japanese medicine, named Kampo. Essentially, Kampo consists of a system of three dichotomies and three substances. The three dichotomies are as follows: Yin-You (yin-yang, positive-negative); Kyo-Jitsu (deficiency-excess); and Netsu-Kan (hot-cold). The three substance categories are qi, blood, and fluid. Qi is fundamental energy of life. Blood and fluid are similar with the common concepts of blood and bodily fluids. In Kampo, it is known that a well-balanced or non-deviated condition of the three dichotomies and three substance concepts leads to a healthy body (3). The International Statistical Classification of Disease and Related Health Problems, the 11th version (ICD-11) approved in 2019 features new traditional medicine. A supplementary chapter of the classification includes symptomatology such as sign, symptoms, and unique findings using traditional medicine diagnostic methods to determine a pattern in traditional medicine that is known as *Sho* in Kampo (4). *Sho* is the central concept and essence of Kampo (5). The Kampo paradigm is completely different from that of modern Western medicine. Four specific diagnostic procedures are used for Kampo medicine, such as inspection, listening and smelling examination, abdominal examination and tongue examination. Especially, the abdominal examination is unique to Japan, and considered to be one of the most important approaches (6). Several studies have reported that the most suitable formula is chosen for a patient after determining *Sho* as a Kampo concept (3, 5, 7, 8). For example, Okita et al. (8) described their observations from a clinical trial that involved selected patients who met the criteria of Kakkonto-*Sho*, and demonstrated that it is more effective than conventional remedies.

In the present article, we report a case of a patient with refractory infertility and early recurrent miscarriage (ERM) who successfully became pregnant with a combination therapy of Kampo medicine, based on *Sho*, and ART. We describe that *Sho* is important for selecting the appropriate Kampo medicine and accumulating evidence to clarify the effectiveness of Kampo with ART.

CASE PRESENTATION

The patient was a 34 year old Japanese woman who had a history of erythema exudative multiforme, and a family history of colorectal cancer in her mother and hypertension in her father. Menarche occurred in the patient when she was 13 years old, after which, she had constant menstrual irregularities. At the age of 30, she got married; she wanted to get pregnant immediately and was worried about her fertility because of her menstrual irregularity. She went to see an obstetrician and gynecologist in a nearby hospital. Although she had been treated with clomiphene or hormone therapy for 2 years at that hospital, pregnancy could not be achieved. She changed her doctors to an infertility specialist at a local clinic. At the first visit, physical examination showed that her height was 163.5 cm and weight was 52.4 kg. Other than a follicle-stimulating hormone (FSH) level of 2.5 mIU/mL (adult female follicular phase: 3.01∼14.72 mIU/mL), hormone testing was normal, with a luteinizing hormone level of 2.3 mIU/mL (adult female follicular phase: 1.76–10.24 mIL/mL), an anti-Müllerian hormone level of 7.79 ng/ml, an estradiol level of 77.7 pg/mL (adult female follicular phase: 28.8–196.8 pg/mL), a progesterone level of 0.1 ng/mL (adult female follicular phase: < 0.28 ng/mL), a testosterone level of 20.1 ng/dL (10.8–56.9 ng/dL), a prolactin level of 12.0 ng/mL (6.1–30.5 ng/mL), a thyroid-stimulating hormone level of 1.33 IU/mL (0.5–5.0 IU/mL), a free T3 level of 3.08 (2.3–4.0 pg/mL) and a free T4 level of 1.13 (0.9–1.7 ng/mL). Immunological blood test was negative for anti-nuclear antibody, anti-sperm antibody and anti- CL-B2GP1 antibody. Analysis of her husband's semen was within normal range. Both hysterosalpingography and hysteroscopy were almost normal, except for a slight bicornuate uterus.

Since transient hyperprolactinemia was observed after 3 years form the first visit of clinic, the patient was prescribed cabergoline for 3 months, after which the level of prolactin decreased. Even though her prolactin level was normalized, she remained unable to conceive. Therefore, the clinic doctor recommended that she undergo ovarian stimulation with recombinant FSH formulation. The first stimulation resulted in anovulation. The second led to an increase in the units of recombinant FSH formulation; as a result, the patient developed ovarian hyperstimulation syndrome. Because of her infertility of unknown origin and repeated failure in ovarian stimulation, she was recommended to try a form of ART such IVF and/or ICSI treatment, to which she agreed. Egg retrieval was performed twice and frozen embryo transfer was done seven times. Although she conceived at the third and seventh attempts, both conceptions resulted in miscarriage in the early pregnancy stage. She desired to improve her physical condition and decided to visit the Kampo clinic at our university hospital. The clinic doctor therefore referred her to our hospital for additional infertility treatment with Kampo, while still continuing ART at her local clinic.

At the first visit, the patient complained of feeling hot. We performed examinations to determine her *Sho*-pattern. The

abdominal examination and pulse examination revealed a strong abdomen and excessive pulse, respectively. We, therefore, made a diagnosis of yang and excess. In addition, we determined that she had blood stasis from the findings such as para-umbilical tenderness, resistance, and lower abdominal resistance, as well as a dark reddish color and sublingual vein distension in the tongue inspection results. We chose a Kampo medicine, keishibukuryogan (KBG, decoction, three times per day before meals), which improves blood stasis, and prescribed for 2 weeks. At the second visit after 2 weeks, she complained of mental instability. Since Kampo finding showed epigastric discomfort and resistance, hypochondriac discomfort and distension, and brisk epigastric aortic pulsation were observed, we considered it to be qi stagnation and added saikokaryukotsuboreito (decoction, three times per day before meals) to KBG to improve her qi. Her hot feeling and mental instability disappeared for a while. After 6 months, she had the feeling of cold extremities, leg edema and tiredness. She described busyness of her work and depressed mood due to the repeated failure of ART. Examinations such as abdominal palpation, tongue inspection and pulse sign revealed a gradual change in the patient's *Sho* pattern from yang and excess to yin and deficiency pattern. Abdominal strength weakened, para-umbilical tenderness was observed, and splashing sound in the epigastric region was noted. Tongue inspection showed a slightly swollen tongue that was pale pinkish in color. Her pulse was weak and floating. We changed the KBG prescription to tokishakuyakusan (TSS, decoction, three times per day before meals) which is used for blood deficiency and fluid disturbance. As qi disturbance was observed during the TSS treatment, kososan (TJ-70, 7.5 g/day, three times per day before meals, TSUMURA & CO, Ltd., Tokyo, Japan) for qi stagnation and rikkunshito (TJ-43, 7.5 g/day, three times per day before meals, TSUMURA & CO, Ltd., Tokyo, Japan) for qi deficiency were added to TSS. Gradually, her feeling of cold extremities, leg edema and tiredness improved. Finally, the patient succeeded in conception after 1.5 years from the beginning of Kampo treatment. At 38 weeks of gestation, she gave birth to a boy by cesarean section because of breech presentation. The newborn had Apgar scores of 8 at 1 min and 7 at 5 min. Her clinical time course from the beginning of ART to conception is shown **Figure 1**.

DISCUSSION

In the present article, we reported a case of a patient with refractory infertility and ERM who successfully became pregnant after Kampo treatment based on *Sho*. The patient had irregular menstruation before marriage. Therefore, she decided to go into fertility treatment immediately after getting married. Finally, she achieved conception and maintained pregnancy 7 years after beginning infertility treatment. We think that it is uncertain whether the addition of Kampo treatment to ART had a direct effect on the conception and pregnancy. However, until Kampo medicine treatment was started, the patient could not give a birth for 5.5 years, even though she was treated with various western treatments. She mentioned that she felt her physical and mental conditions had improved after starting a Kampo medicine. Therefore, we believe that additional Kampo medicine to ART treatment was effective for our patient, even though it is uncertain whether it had direct effects on the reproductive organs.

There are several reports, including systematic reviews, on the effects of traditional Chinese medicine (TCM) for infertile women (9–13). Most of these demonstrated the effectiveness and safety of TCM, although a small number of trials did not show effectiveness. Lee et al. performed a systematic review in 2015 and showed a better clinical pregnancy rate (RR 1.74, 95% CI 1.56–1.94) with herbal medicine (13). For infertile women undergoing IVF, Cao et al. (9) reported significant effects of TCM on clinical pregnancy rate (OR 2.04, 95% CI 1.67–2.49) and ongoing pregnancy rate (OR 1.91, 95% CI 1.17–3.10). Although these two studies demonstrated that TCM is effective for infertile women with or without the use of Western medicine, there were several differences between them such as contents of medicine, duration of medication and method of administration. Therefore, at present, there are some limitations with TCM treatment for infertility, especially regarding a lack of uniform standards.

Alternatively, Kampo is a unique, traditional Japanese herbal medicine, with *Sho* in its central concept (5). In the present case, at the first visit to our hospital, the patient was diagnosed as yang and excess pattern, and blood stasis. According to the diagnosis, we prescribed KBG. Since her pattern changed from yang to yin pattern after 6 months, we changed her Kampo medicine from KBG to TSS. Both KBG and TSS are typically prescribed for the treatment of menstruation-related symptoms such as irregularities, premenstrual syndrome, dysmenorrhea and menopausal syndrome. As shown in **Table 1**, KBG is used for yang and blood stasis, and TSS is used for yin, blood deficiency, fluid disturbance and blood stasis. The important points are that the *Sho*-pattern needs to be carefully followed during the infertility treatment, and the change of its pattern should not be missed.

Previous studies have reported that several Kampo medicines were effective in women with infertility. Ushiroyama demonstrated clinical usefulness of Unkei-to in anovulatory and/or infertile women (14). It is suggested that Unkei-to targets at the hypothalamus and the pituitary glands and that its mechanism involves adjusting the gonadotropin level *in vivo* to a physiologically appropriate level. Otani et al. reported the usefulness of hachimijiogan for treatment of hyperprolactinemic infertile women with a pituitary microadenoma (15). In their report, although the patient was resistant to bromocriptine, she succeeded in having a normal pregnancy and delivery with hachimijiogan. Usuki et al. (16) showed that TSS improved luteal insufficiency in women but did not affect the hormonal levels with normal menstrual cycles. As a molecular mechanism for ovarian function, it has been reported that TSS stimulated progesterone and estradiol-17 beta in rat granulosa cells (17). Regarding the uterus, Terawaki et al. (18) reported that the ameliorating effects of TSS in a rat model of implantation failure may involve the alleviation of decreased leukemia inhibitory factor production derived from the endometrial gland, and decidualization dysfunction. Certainly, in the current case,

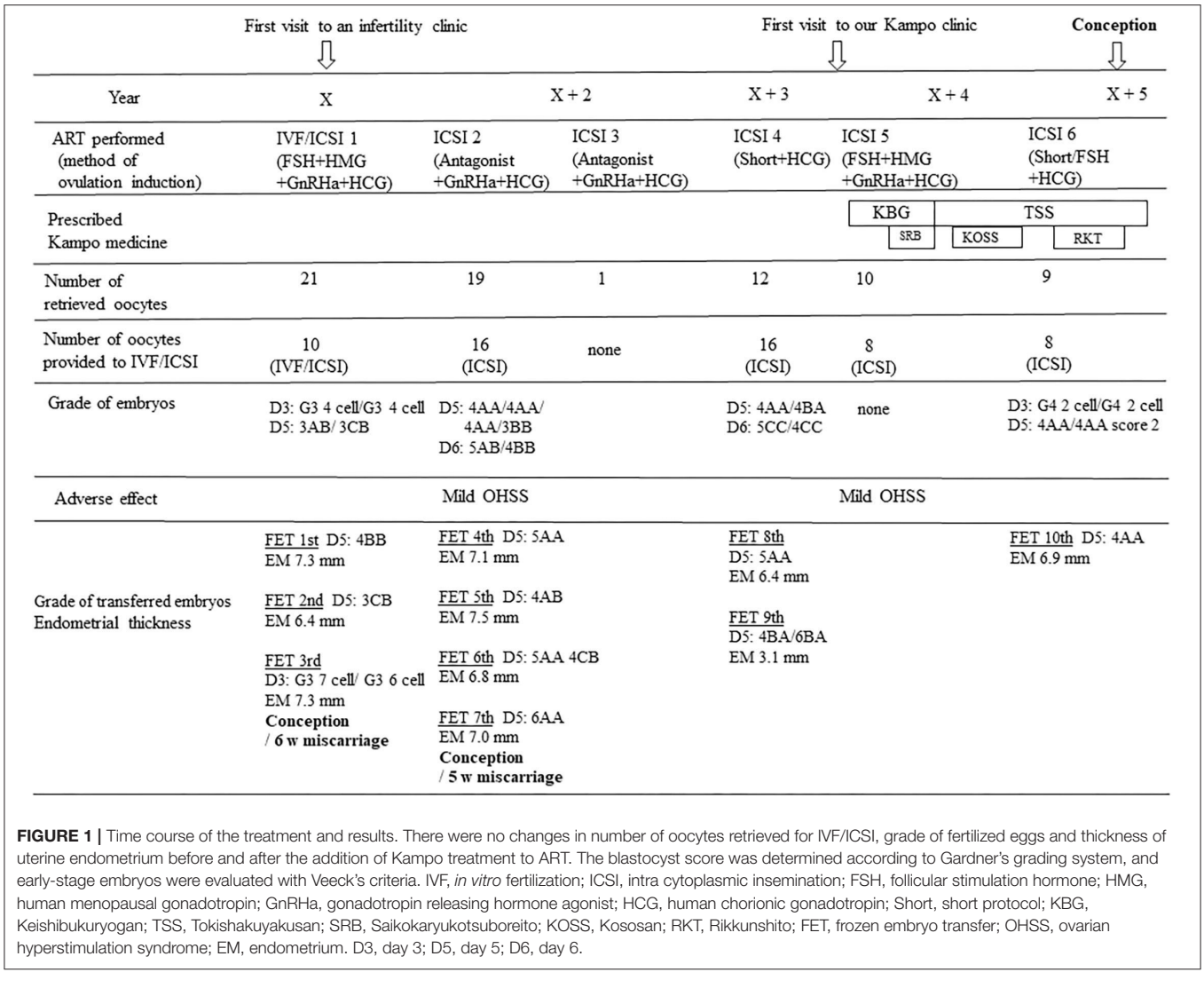

FIGURE 1 | Time course of the treatment and results. There were no changes in number of oocytes retrieved for IVF/ICSI, grade of fertilized eggs and thickness of uterine endometrium before and after the addition of Kampo treatment to ART. The blastocyst score was determined according to Gardner's grading system, and early-stage embryos were evaluated with Veeck's criteria. IVF, *in vitro* fertilization; ICSI, intra cytoplasmic insemination; FSH, follicular stimulation hormone; HMG, human menopausal gonadotropin; GnRHa, gonadotropin releasing hormone agonist; HCG, human chorionic gonadotropin; Short, short protocol; KBG, Keishibukuryogan; TSS, Tokishakuyakusan; SRB, Saikokaryukotsuboreito; KOSS, Kososan; RKT, Rikkunshito; FET, frozen embryo transfer; OHSS, ovarian hyperstimulation syndrome; EM, endometrium. D3, day 3; D5, day 5; D6, day 6.

TABLE 1 | Prescriptions and components of KBG and TSS.

	Keishibukuryogan (KBG)	**Tokishakuyakusan (TSS)**
Components	(1) Keihi = Cinnamon Bark 4 g (2) Bukuryo = Poria Sclerotium 4 g (3) Shakuyaku = Peony Root 4 g (4) Botanpi = Moutan Bark 4 g (5) Tounin = Peach Kernel 4 g	(1) Toki = Angelica Root 3 g (2) Senkyu = Cnidium Rhizome 3 g (3) Shakuyaku = Peony Root 4 g (4) Bukuryo = Poria Sclerotium 4 g (5) Byakujyutu = Atractylodes Rhizome 4 g (6) Takusya = Alisma Tuber 4 g
Sho	yang and excess, blood stasis	ying and deficiency, fluid disturbance, blood deficiency, blood stasis
Target group	hot flashes, tenderness in para-umbilical area	cold extremities, edema, dizziness, splashing sound in epigastric region, tenderness in para-umbilical area
Indications	menstrual irregularity, dysmenorrhea, menopausal syndrome	anemia, menstrual irregularity, dysmenorrhea, menopausal syndrome, infertility, various symptoms during pregnancy such as edema, recurrent miscarriage, and abdominal pain

we suspect that TSS impacted not only implantation but also miscarriage prevention.

It is not clear why the *Sho* pattern in our patient changed 6 months after starting treatment with KBG. Imai et al. (19) reported that lower educational background, longer duration of infertility (>2 years), being non-permanent worker, harassment experience in the workplace, and lack of support within one's company were identified as risk factors for stress after initiating infertility treatment. It is possible that infertility itself and infertility treatment are associated with increased distress. Especially, insurance medical treatment is currently not available for ART treatment in Japan, and continuing ART treatment for long periods of time can be stressful from an economical perspective. We suppose that the patient in the current report might have felt depressed and stressed due to recurrent unsuccessful ART results.

This case report had a few limitations. First, we showed only one case. The repeatability of KBG and TSS on infertility should be warranted through rigorously designed clinical trials based on *Sho*. Second, the different mechanisms of actions of KBG and TSS remain unclear. Further study will be needed for characterizing the mechanism for whole body and reproductive organs such as uterus and ovary. Third, although *Sho* in Kampo and *Zheng* in TCM were derived from the same word, they have acquired different meanings (7, 20–22). In this case report, it should be noted that we described *Sho* in Kampo. On the other hand, there are strengths in this report. One is, since we are specialized in Kampo medicine, we could accurately diagnose *Sho* of the patient. The other is, we could coordinate medical treatment performed by Kampo medicine specialists and a doctor specialized in reproductive medicine in the patient.

In this case, in particular, we emphasized the value of *Sho* evaluation in the intractable condition and could show how *Sho* changed in the treatment course over the years. *Sho* is an indicator of evaluation for not only individual organs, but also general condition. Kampo medicine contributed to the correction of her systemic condition. As a result of microenvironment improvement, functional recovery of the reproductive organs such as uterus and/or ovary might have been achieved.

CONCLUSIONS

The combination of Kampo with Western medicine such as ART for infertility treatment is considered effective for achieving conception in women with refractory infertility and ERM. In addition, it is important to note that evaluation of the *Sho*-patterns, via examinations such as abdominal palpation, tongue inspection and pulse signs, is essential for selecting the appropriate Kampo medicine. If medical doctors prescribing Kampo can diagnose the patient's *Sho*, the efficiency of treatment might increase. At present, evidence that clarifies *Sho*-patterns in Kampo medicine is not enough concerning its effectiveness in combination with ART. Further studies are needed to accumulate sufficient evidence.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Written informed consent was obtained from the participant for the publication of this case report.

AUTHOR CONTRIBUTIONS

All authors provided contributions to the conception, design, drafting, and critical revision of the study. All authors have read the manuscript and approved it for submission for publication.

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CASE REPORT
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Case Report: Kampo Medicine for Non-tuberculous Mycobacterium Pulmonary Disease

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Background: While the number of pulmonary tuberculosis cases has decreased, increase in non-tuberculous mycobacterium pulmonary disease (NTM-PD) is a global problem. Guideline-based therapy for NTM-PD sometimes causes complications that prevent treatment completion, and there are many cases of relapse even if the treatment can be completed. In addition to antibacterial treatment, care of host risk factors, such as aging, lean physique and immunosuppressive state, is also very important for the management of NTM-PD. In Japan, Kampo medicine, a traditional Japanese herbal formulation, used alone or in combination with standard multidrug therapy for NTM-PD, has been found to be effective for such cases.

Case Presentation: A 77-year-old lean woman had been diagnosed with *Mycobacterium intracellulare* pulmonary infection 6 years earlier, and had received the standard multidrug treatment 5 years later at a former hospital due to worsening of her symptoms of cough, breathlessness and hemoptysis. However, the treatment was discontinued within a year due to the development of adverse events. She refused the guideline-based antibacterial treatment, and asked for Kampo medicine instead. Bukuryoshigyakuto was subsequently prescribed, which led to cough and sputum, especially hemosputum, being well controlled. With 3 years of Kampo medicine treatment, she gained weight and her hemosputum disappeared. High-resolution computed tomography images showed improvement in her lung condition, and her sputum smear culture was negative for acid-fast bacillus.

Conclusion: Various kinds of Kampo medicines have been used empirically for NTM-PD in Japan. A literature review from 1992 to 2020 showed that hozais, in particular, seem to be key drugs for the treatment of host NTM-PD risk factors. Kampo medicines can contribute to comprehensive treatment for NTM-PD management that does not rely solely on antibacterial drugs.

Keywords: non-tuberculous mycobacterium-pulmonary disease (NTM-PD), Kampo medicine, hozai, bukuryoshigyakuto, hochuekkito

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INTRODUCTION

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms that live in soil and natural water sources (1). NTM commonly cause pulmonary disease (NTM-PD), which is sometimes intractable and difficult to manage (1, 2). The incidence rate of NTM-PD in Japan in 2014 was 14.7 per 100,000 people, representing an increase of 2.6 times compared to the results of a 2007 survey (3). Although basic epidemiological data are lacking in most regions, the increase in NTM-PD cases seems to have become a global problem (4–6). Over 180 NTM species have been discovered to date, only some of which are reported to cause pulmonary disease (1). The most commonly isolated species are the *Mycobacterium avium-intracellulare* complex (MAC) and *M. abscessus* complex (*M. abscessus* subsp. *abscessus*,*M. abscessus* subsp. *massiliense*,*M. abscessus* subsp. *bolletii*) (1), and a recent study in Japan showed that MAC accounts for nearly 90% of all NTM-PD cases (3).

Standard treatment regimens for NTM-PD include recommended combinations of drugs, such as macrolides and others, which need to be continued for a long period at least 1 year after culture negative was confirmed (2). This leads to the risk of treatment non-compliance; additionally, there are some cases in which treatment cannot be completed due to the occurrence of toxic adverse events (7). It was previously reported that even when treatment is undertaken in line with guidelines, treatment success rates for MAC range over 32–65% (8). Further, there are some cases that develop refractory disease with high mortality and morbidity (1). New therapeutic strategies, such as amikacin liposome inhalation, are now recommended for refractory NTM-PD cases (9), although the therapeutic options remain limited. The management of NTM-PD is therefore thought to require a holistic and multidisciplinary strategy, and not just antibiotic treatment, to achieve better outcomes (10).

Kampo medicine is a traditional Japanese herbal medicine system that originated from traditional Chinese medicine. Kampo medicines were earlier taken as a decoction. After the medicines began being supplied in dried extract form instead of the decoction in 1976, the number of people who took Kampo medicines rapidly increased in Japan. Currently, there are 148 formulas and about 150 individual herbs covered by the Japanese insurance program. Kampo medicines are traditionally used for a wide range of symptoms and diseases. The symptoms treated by Kampo medicines vary widely, and include neuralgia, arthralgia, chronic headache, shoulder stiffness, frailty and sensitivity to cold. The diseases for which they are prescribed are also diverse, and Kampo medicines are also used for respiratory diseases such as asthma (11), chronic obstructive pulmonary disease (COPD) (12) and MAC (13). Hochuekkito, a Kampo medicine, is used as an adjunct to conventional treatment for general malaise, appetite loss and physical exhaustion, and a pilot open-label quasi-randomized controlled study used the medicine in patients with progressive pulmonary MAC disease despite standard antibiotic therapy over 1 year, who were persistently culture-positive or intolerant to antibiotic therapy (13). In Japan, although guideline-based treatments have been prioritized, Kampo medicine is considered for refractory cases and cases in which it is difficult to continue antibiotic therapy due to the development of adverse effects to drugs. Therefore, various kinds of Kampo medicines have been used for NTM-PD, such as hochuekkito, ninjinyoeito, saikanto, and chikuyosekkoto (14). Above all, hochuekkito and ninjinyoeito are thought to have contributed as "hozai"s, i.e., tonic formulas, that strengthen the body and restore depleted qi and "blood" (used here as a term of traditional medicine and described later), which is one of the key principles of Kampo medicine (15). This concept is similar to the principle of addressing host risk factors of NTM-PD, such as aging and the associated frailty and immunosuppression. Another Kampo medicine that is considered as a hozai is bukuryoshigyakuto, which consists of *Ginseng radix*, *Aconiti tuber*, *Glycyrrhizae radix*, *Poria cocos* and *Zingiber siccatum*.

Here, we present a case of NTM-PD that was treated with bukuryoshigyakuto. Additionally, we researched and summarized previous similar reports to clarify the usefulness of Kampo medicines for NTM-PD.

CASE DESCRIPTION

A 77-year-old woman had been diagnosed with *Mycobacterium intracellulare* infection at another hospital 6 years earlier. Her symptoms of cough, breathlessness and hemoptysis worsened over 5 years without standard treatment for NTM, and combination drug therapy with macrolides (600 mg clarithromycin, 450 mg rifampicin and 750 mg ethambutol) was started 5 years after her initial diagnosis. A few months to a year later, she gradually developed adverse effects to drug therapy, such as ambulation difficulty due to numbness in her legs, weight loss and visual impairment, with persistence of cough and bloody phlegm. Hence, she decided to seek alternative treatment and was referred to our hospital. She did not have any other medical or relevant family history. She never smoked or drank alcohol. At her initial visit to us, she was 144.6 cm tall, weighed 30.0 kg, and was remarkably emaciated. She came to us in a wheelchair. Although her percutaneous oxygen (SpO_2) saturation was 96% at rest, she could not have a long conversation, and could not even adopt the supine position, because of coughing with production of a large amount of sputum and breathlessness. Coarse crackles were heard on auscultation. A high-resolution computed tomography (HRCT) scan performed at the first consultation showed bronchiectasis that was predominantly in the middle lobe and lingular segment, bilateral centrilobular lesions and dorsal predominant bronchiolitis (**Figure 1A**). Her laboratory data were as follows: white blood cell count: 5,380 /μl; neutrophils: 4,180 /μl; lymphocytes: 678 /μl; C reactive protein level: 0.19 mg/dl; albumin: 3.6 g/dl; and hemoglobin: 10.8 g/dl (**Table 1**). According to the case notes of her previous doctor, *M. intracellulare* had been identified in her sputum, although

Abbreviations: AFB, acid-fast bacillus; BMI, body mass index; COPD, chronic obstructive pulmonary disease; HRCT, high-resolution computed tomography; MAC, *Mycobacterium avium-intracellulare* complex; NTM, nontuberculous mycobacteria; NTM-PD, NTM commonly causes pulmonary disease; QOL, quality of life; SpO_2, percutaneous oxygen; [TM1], [traditional medicine module 1].

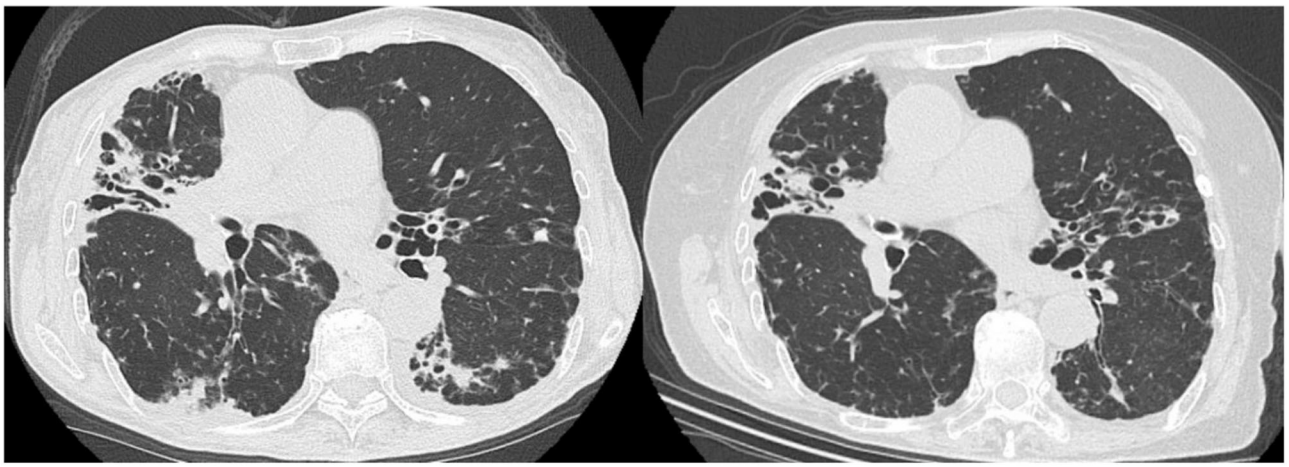

FIGURE 1 | High-resolution computed tomography images before **(A)** and after **(B)** bukuryoshigyakuto treatment. **(A)** The image taken at the first visit at our hospital in 2015 showed middle lobe and lingular segment predominant bronchiectasis, bilateral centrilobular lesions, and dorsal predominant bronchiolitis. **(B)** In 2018, bilateral centrilobular lesions, consolidation, and dorsal predominant bronchiolitis lesions were improved, though the middle lobe and lingular segment bronchiectasis had no change.

her sputum was acid-fast bacillus (AFB) smear-negative and culture-negative at the time of her initial visit to our hospital. We tried to recommend guideline-based treatment in view of the fact that her AFB status had become negative with standard treatment for about 1 year while considering her previous adverse events to therapy. However, the patient and her family stubbornly refused antibiotic-based treatment and preferred treatment with Kampo medicines. She had remarkable emaciation, with a body mass index (BMI) of 14.4 kg/m^2 and poor nutritional status, and she was totally exhausted due to persistent respiratory symptoms and adverse effects of therapy. She also suffered from serious appetite loss and insomnia associated with symptoms of NTM-PD. Kampo evaluation showed that her limbs were cold, pulse was weak, and abdominal strength was weak. We thus prescribed bukuryoshigyakuto based on diagnosis of yin and deficiency patterns. Hence, a bukuryoshigyakuto (*Panax ginseng* 1g, *Aconiti tuber* 1g, *Glycyrrhizae radix* 2g, *Poria cocos* 5g and *Zingiber siccatum* 2g) decoction (to infuse a total of 11 g of each herb with 400 ml of water for 30–40 min to make 200 ml, and to take in two divided doses) was started after a medical examination based on Kampo principles, while discontinuing the current treatment with clarithromycin, rifampicin and ethambutol. Within a few months, her eyesight improved, her gait disturbance also improved, and she no longer needed to use a wheelchair. Her appetite returned, and her hemoptysis and sputum gradually decreased. During the course of her treatment, she stopped taking bukuryoshigyakuto for a while due to the complexity of the decoction. She again recognized an increase in the hemoptysis and restarted the medication, and since then has never stopped it. Her HRCT image findings also improved. Although the middle lobe and lingular segment bronchiectasis showed no changes, bilateral centrilobular lesions and dorsal predominant bronchiolitis improved significantly (**Figure 1B**).

TABLE 1 | Laboratory data before and after bukuryoshigyakuto treatment.

	At the first visit	3 years later
Body weight (kg)	30.0	42.0
WBC (/ml)	5,380	4,600
Neutrophils (/μl)	4,180	2,880
Lymphocytes (/μl)	678	1063
CRP (mg/dl)	0.19	0.07
Total protein (g/dl)	8.0	7.9
Albumin (g/dl)	3.6	3.9
Hemoglobin (g/dl)	10.8	11.4

It compared the body weight and the main laboratory data before and 3 years after bukuryoshigyakuto. WBC, white blood cell; CRP, c-reactive protein.

Her body weight increased from 30 to 42 kg within 3 years after starting bukuryoshigyakuto. Her laboratory data also improved, to a neutrophil count of 2,880 /μl, lymphocytes of 1,063 /μl, albumin level of 3.9 g/dl and hemoglobin level of 11.4 g/dl (**Table 1**). Her shortness of breath, hemoptysis and sputum also finally improved, although she had some residual cough and slight numbness in her legs. Currently, continuing outpatient visits and bukuryoshigyakuto therapy have maintained her AFB smear and culture negative status.

DISCUSSION

The current report presents bukuryoshigyakuto treatment of an NTM-PD patient who discontinued guideline-based antibiotic therapy due to adverse events. As mentioned by Ali, management of NTM-PD, which is a debilitating, often refractory, progressive lung disease, therapy for which must

be customized beyond antimicrobials to encompass various kinds of medical wisdom (10). In Japan, Kampo medicines are occasionally considered in NTM-PD cases with cough and expectoration who cannot continue standard therapy due to adverse events or in whom standard therapy is not expected to have adequate effect. The purpose of this report was to introduce the usefulness of hozais, which restore depleted qi and blood[TM1], which is one of the main principles behind Kampo medicine, including bukuryoshigyakuto for NTM-PD. The International classification of Diseases 11th Revision i.e., ICD-11 now included traditional medicine in chapter 26, and [TM1] refers to Traditional Medicine conditions -Module I (16). The [TM1] designation is used for traditional medicine diagnostic category in order to be clearly distinguishable from conventional medicine concepts.

In general, some natural compounds, including Kampo medicines, have the potential to upregulate host immunity, and it is expected that this effect, rather than their bactericidal effects, is useful in the treatment of NTM (17). In fact, Chinese herbal medicines containing *Astragalus membranaceus*, *Radix Scutellariae*, *Radix Stemonae*, *Rhizoma Salviae Miltiorrhizae* and *Radix Euphorbiae Fischerianae* were used as adjuvant treatment to chemotherapy for multidrug-resistant tuberculosis from regard of improving immune function (18). The host risk factors for NTM-PD are well identified. Structural lung diseases, such as bronchiectasis, COPD and interstitial lung disease, are known to predispose an individual to developing NTM-PD (19–21). The onset of NTM-PD has also been recognized in persons with no previously diagnosed underlying risk factors, the so-called "Lady Windermere" syndrome that was named after a character in an Oscar Wilde novel (22, 23). The majority of subjects are female, post-menopausal, taller and thinner than average, and their susceptibility is related to hormonal factors, connective tissue abnormalities and low adiposity (24). Elderly age is also a pivotal risk factor for NTM-PD (1), and actually, the incidence of NTM-PD has increased in the super-aging society of Japan (25). Furthermore, frequent use of immunosuppressant medications, such as corticosteroids and biological therapy for collagen diseases such as rheumatoid arthritis, is also spurring an increase in NTM-PD (26). These host risk factors can sometimes overlap; for example, an old thin woman with rheumatoid arthritis treated with biological immunosuppressants might represent an ideal candidate for development of NTM-PD. The role of hozai therapy is to improve the host risk factors of NTM-PD.

Hozais, such as hochuekkito and ninjinyoeito, include a group of formulas that supposedly invigorate patients who have lost physical and mental energy due to various reasons (15), such as patients with cancer, refractory inflammatory diseases and chronic infectious diseases who are mentally and physically exhausted. NTM-PD and COPD are typical examples of these diseases, and the patients often experience an impaired quality of life (QOL) due to persistent respiratory and depressive symptoms (27, 28). In this regard, hozais could be very useful for supporting the patients' QOL. Hochuekkito is known to have immunomodulating effects by increasing serum interferon-gamma levels (29–31), and it is thought to be useful for infectious and inflammatory diseases (32). The therapeutic effect of hochuekkito on MAC is a good example (13). Enomoto et al. showed that hochuekkito is an effective adjunct to conventional therapy in patients with progressive NTM-PD (13). In their study, although none of the patients achieved sputum conversion, the number of colonies in sputum generally remained stable in the Hochuekkito group, while it tended to increase in the control group (13). Further, the hochuekkito group tended to have an increase in body weight and serum albumin levels compared with their respective values in the control group (13). Ninjinyoeito, which is another hozai for such as frailty (33), has also been used for NTM-PD. Nogami et al. reported the efficacy of ninjinyoeito in a patient with NTM-PD due to *Mycobacterium fortuitum,* who did not improve despite receiving guideline-based therapy for 2 years (34). His symptoms, such as cough, hemoptysis and general malaise, gradually improved with ninjinyoeito therapy, and 10 months later his sputum converted to smear negative (34).

In the present report, bukuryoshigyakuto was used for NTM-PD. This medicine is used in patients with yin and deficiency patterns. Bukuryoshigyakuto is also considered a hozai, and bukuryoshigyakuto-sho is recognized more deficiency than hochuekkito-sho or ninjinyoeito-sho, where "Sho" is the

TABLE 2 | Comparison of previously reported data pre- and post- Kampo medicine treatment by hozai.

	Age (year)	Sex	Add on/alone	Administration period of Kampo medicine (month)	Changes in parameter with treatment					
					BW (kg)	Alb (g/dl)	Hb (g/dl)	Ly (/μl)	Neu (/μl)	CRP (mg/dl)
Hochuekkito										
Ashino and Hattori (45)	80	F	add on	12	+1	+0.3	+1.1	+405	−1,263	−1.4
Enomoto et al. (13)	70*	F7:M2	add on	6	+0.4*	+0.2*				+0.06*
Ninjinyoeito										
Nogami et al. (34)	72	M	alone	15	+2		+1.1			
Bukmyoslugyaku	77	F	alone	36	+12	+0.3	+0.6	+385	−1,300	−0.12

*Body weight, albumin, hemoglobin, lymphocytes, neutrophils, and c-reactive protein were compared. In second line, hochuekkito group, *showed median quoted from Enomoto et al. (13). BW, body weight; Alb, albumin; Hb, hemoglobin; Ly, lymphocyte; Neu, neutrophil; CRP, c-reactive protein.*

diagnosis of the patient's signs and symptoms comprehensively based on theories of Kampo medicine (35). Her general fatigue and appetite loss due to NTM-PD are considered good indications for hochuekkito and ninjinyoeito. However, her condition with emaciation, coldness of her limbs and weakness of her pulse was thought to be in yin and deficiency patterns, and to be more suitable for bukuryoshigyakuto than hochuekkito and ninjinyoeito. With this treatment, her appetite recovered and the cold sensation in her limbs gradually improved. Subsequently, her respiratory symptoms drastically decreased, and sputum culture remained AFB negative. According to tests for AFB, although previous treatment with antibiotics seemed to have been effective against *M. intracellulare*, it did not improve her QOL.

Bukuryoshigyakuto consists of *Panax ginseng*, *Aconiti tuber*, *Glycyrrhizae radix*, *Poria cocos* and *Zingiber siccatum*. Bukuryoshigyakuto is based on shigyakuto, which consists of *Aconiti tuber*, *Glycyrrhizae radix* and *Zingiber siccatum*. Shigyakuto is also used for patients with a yin deficiency pattern. Zang et al. reported that shigyakuto had protective effects, improving the microcirculatory disturbances induced by endotoxins in rat mesentery (36), and it could be said that improvement of microcirculatory disturbance is one of the main effects of drugs used for the treatment of a yin deficiency pattern. The effect might be associated with "ompo", which is a method of treating deficiencies associated with cold[TM1] patterns using warming-tonifying formulas. Shigyakuto has also been shown to have antiviral activity in mice infected with herpes simplex virus type 1 through the activation of CD8+ T cells (37). *Zingiber siccatum* is a pivotal constituent herb of both shigyakuto and bukuryoshigyakuto. 6-Shogaol, one of the components of *Zingiber siccatum*, was recently reported to have antibiofilm activity against *Candida albicans* (38). NTM is an environmental organism inhabiting soil and water that also forms a biofilm (39). Although there are differences between fungi and acid-fast bacilli, 6-shogaol might be effective in inhibiting biofilm formation.

Bukuryoshigyakuto also includes Ginseng radix, which is also a component of hochuekkito and ninjinyoeito. Kampo formulas containing *Ginseng radix* and *Astragali radix* are called "Jingizai", and they are typical hozais like hochuekkito and ninjinyoeito. *Ginseng radix* (*Panax ginseng*), in particular, has been widely used in Kampo formulas. The clinical efficacy of *Panax ginseng* was well described in various kinds of studies (40). In a meta-analysis of 12 randomized controlled trials, Bach et al. reported the efficacy of ginseng supplements in alleviating fatigue (41). Another study showed that *Panax ginseng* improved respiratory muscle strength and lung function in COPD patients (42). It was also reported that Chinese herbal medicines including ginseng improved the results of 6-min walking tests in stable COPD patients (43). Further, ginsenoside Rg1, one of the saponin groups present in *Panax ginseng*, stimulates the proliferation of lymphocytes, and ginsenoside might enhance cellular immune function (44).

We reviewed the literature using PubMed and Ichushi-Web, which is a database of the Japanese Medical Abstracts Society, to identify articles which Kampo medicines were used for NTM-PD published till now. Although we and other respiratory clinicians do use hozais for NTM-PD patients on a daily basis, unfortunately, there are less articles than we expected. This limited number of patients could also be due to the fact that clinicians might not report their experiences with medicines that do not have global recognition. We performed a comparison of data before and after administration of Kampo medicines by hozai in previous cases of NTM-PD. As shown in **Table 2**, hochuekkito (13, 45), ninjinyoeito (34) and bukuryoshigyakuto at least seemed to increase body weight and albumin level. It is well known that chronic inflammatory pulmonary diseases result in weight loss and poor nutritional status (12). Hozais could be useful by preventing these symptoms.

Our study has some limitations in terms of clarification of the usefulness of Kampo medicines for NTM-PD. The current study is just an introduction to cases of use of Kampo medicine for NTM-PD. We admit that many doctors use Kampo medicines in their daily practice, although there are very few case reports describing this. Even if there are case reports, the data are not standardized, making them difficult to compare and summarize. Hence, it is necessary to conduct a nationwide multicenter survey to understand the use of Kampo medicines for NTM-PD, including a retrospective observation study. For further investigation, a randomized controlled study will be the next step to investigating the usefulness of Kampo medicines for NTM-PD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

TS, RA, TK, and HN designed the study. TS and KU collected the data. TS, TK, and KU analyzed the data. TM advised about Kampo medicines. TS and HN wrote the manuscript. All authors read and approved the final manuscript.

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REVIEW
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Ghrelin Enhancer, the Latest Evidence of Rikkunshito

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Rikkunshito is a Japanese herbal medicine (Kampo) that has been attracting attention and researched by many researchers not only in Japan but also worldwide. There are 214 rikkunshito articles that can be searched on PubMed by August 2021. The reason why rikkunshito has attracted so much attention is due to an epoch-making report (Gastroenterology, 2008) discovered that rikkunshito promotes the secretion of the orexigenic peptide ghrelin. Since then, many researchers have discovered that rikkunshito has a direct effect on the ghrelin receptor, GHS-R1a, and an effect of enhancing the ghrelin signal to the brain. Additionally, a lot of evidence that rikkunshito is expected to be effective for various gastrointestinal diseases have also been demonstrated. Numerous basic and clinical studies have suggested that rikkunshito affects (i) various discomforts caused by anticancer drugs, gastroesophageal reflux disease, functional dyspepsia, (ii) various stress-induced anorexia, (iii) hypophagia in the elderly, and (iv) healthy lifespan. In this review, as one who discovered the ghrelin enhancer effect of rikkunshito, we will review the research of rikkunshito so far and report on the latest research results.

Keywords: ghrelin, GHS-R, anorexia, rikkunshito, Kampo, stress, aging

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RIKKUNSHITO

Rikkunshito is one of the prescriptions described in the old medical book *Return of Spring from All Kinds of Diseases* compiled by Kyoenken in 1587. Rikkunshito comprises eight herbal medicines, *Atractylodis lanceae rhizoma, Ginseng radix*, *Pinelliae tuber, Hoelen, Zizyphi fructus*, *Aurantii nobilis pericarpium*, *Glycyrrhizae radix* and *Zingiberis rhizoma*. It was used for patients with gastrointestinal weakness, loss of appetite, epigastrium, tiredness, anemia, and chills in the limbs. In Japan, it is an insurance coverage drug for gastritis, gastric atony, gastroptosis, indigestion, loss of appetite, stomach pain, and vomiting, which is covered by a doctor's prescription. The first high-quality evidence is a multicenter comparative study of TJ-43 rikkunshito for indefinite gastrointestinal complaints, such as chronic gastritis by Harasawa et al. (1). Additionally, a multicenter, double-blind study using rikkunshito has recently been conducted, including proton pump inhibitor refractory non-erosive reflux disease (NERD, $n = 242$) (2) and functional dyspepsia ($n = 192$) (3), and it was proven to be effective for them. Rikkunshito was compared to the placebo treatment group, and the degree of improvement of total and the acid-related dysmotility symptom scores of the frequency scale for the symptoms of gastroesophageal reflux disease (FSSG) after the 8-week treatment was significantly greater in the rikkunshito group than in the placebo group. Rikkunshito also significantly increased the global assessment of overall treatment efficacy in functional dyspepsia patients and improved upper gastrointestinal symptoms after 8 weeks,

especially postprandial fullness/early satiety and bloating. Thus, except acid secretion inhibitors and acotiamide, there is poor evidence of drugs for digestive system complaints so far, so the accumulation of clear evidence by Japanese Kampo medicine is an important event.

Additionally, rikkunshito's mechanisms of action have been extensively studied in detail. Rikkunshito has been proven to enhance gastric emptying (4–6) and promote adaptive relaxation reaction (6–10) of the stomach from basic and clinical aspects. These actions are not merely single pharmacological actions, such as increased gastric motility or decreased gastric acid secretion, but have the characteristic of enhancing the overall function of the stomach. Thus, it can be said that the mechanism of action of rikkunshito is significantly different from that of new drugs with a single pharmacological action.

Furthermore, the epoch-making evidence that rikkunshito was made known to global gastroenterologists was believed to be discovering the ghrelin-enhancing effect of the orexigenic peptide by rikkunshito (11). Inspired by the common characteristics of patients after taking rikkunshito, we discovered that rikkunshito may enhance the action of ghrelin and proved the first evidence. After that, many researchers proceeded with further detailed research, and rikkunshito enhanced the gene expression of the ghrelin receptor GHS-R1a (12) and that the signal transduction of ghrelin was enhanced by improving the binding between ghrelin and GHS-R1a (13).

This review focuses on the effects of rikkunshito on ghrelin and describes the latest evidence and the following possibilities regarding the potential treatment of rikkunshito for various diseases.

ACTION AND MECHANISM ON GHRELIN

What Is Ghrelin?

Ghrelin is a unique peptide with 28 amino acids and an n-octanoyl group. Its main production site is localized to X/A-like cells in the gastric mucosa (14). The hunger signal from periphery is increased by the production of ghrelin, for example, hypoglycemia. As a reflection, it is also increased in the blood. The orexigenic activity of ghrelin is triggered by the serine 3-acyl modification of octanoic acid (acylated ghrelin). This acylation process is catalyzed by the gastric membrane-binding protein, ghrelin *O*-acyltransferase (GOAT) (15). However, acylated ghrelin released into tissues or blood is immediately metabolized by liver-derived butyrylcholinesterase (16) to des-acyl ghrelin, which has no orexigenic effect (**Figure 1**). A difficult point in measuring the active form of acylated ghrelin in clinical research is the instability of acylated ghrelin. The half-life after intravenous injection of acylated ghrelin is 8 min in rats (17). In the case of animal experiments, it should be conducted within 3–5 min from blood sampling to centrifugation by multiple researchers before adding concentrated hydrochloric acid and freezing in liquid nitrogen. A deep freezer at −80°C is optimal for storage. However, it is not easy to freeze within this time after collecting blood from a patient. Therefore, if the conditions at the time of blood collection are different, it is expected that the acylated ghrelin value will also differ significantly from facility to facility or from day to day.

The target of the ghrelin ligand is growth hormone secretagogue receptor 1a (GHS-R1a), a 7-transmembrane G protein-coupled receptor composed of 366 amino acid residues. GHS-R1a is present in the terminal of the vagal afferent nerve, pancreatic cells, spleen, myocardium, bone, fat, thyroid gland, adrenal gland, and immune cells (18, 19). Particularly, it is also densely expressed in the hypothalamic nucleus in the central nervous system (18, 19). The peripheral to the central ghrelin signal is initiated by the binding of GHS-R1a present at the vagal nerve terminal in the gastric mucosa to acylated ghrelin, and the signal to the central nervous system is transmitted. Ghrelin bound to GHS-R1a transmits its starvation signal via the solitary nucleus (NTS) of the medulla oblongata to neuropeptide Y (NPY)/Agouti-related peptide (AgRP) neurons, which are localized in the arcuate nucleus of the hypothalamus and the signal is transmitted to higher centers. Ghrelin stimulates NPY/AgRP neurons and promotes the production of NPY/AgRP peptides and proceeds to induce appetite (20) and promotes gastrointestinal fasting contraction via the vagus nerve efferent pathway (21, 22). GHS-R1a is also localized in the ventral tegmental area (23) and hippocampus (24). Thus, in addition to the action on appetite and energy metabolism, ghrelin may also be involved in cognition and memory and is thought to play an essential role in maintaining neuropsychiatric homeostasis in both peripheral and central tissues.

Serotonin and the adrenaline system play a major role in energy metabolism and regulate the secretion of ghrelin-producing cells, X/A-like cells in the gastric mucosa (11, 25–27). Particularly, intraperitoneal (IP) administration of an agonist of serotonin 2B receptor (5-HT_{2B}R) BW723C86 or an agonist of 5-HT_{2C}R, meta-chlorophenyl piperazine (mCPP), to rats significantly reduces acylated ghrelin levels in peripheral blood (11, 25). Administration of the 5-HT_{2B}R or $_{2C}$R antagonists significantly reversed decreases in peripheral ghrelin and food intake in several disease models (11, 28–30). Cell damage releases large amounts of serotonin after chemotherapy such as cisplatin administration, but it is easy to imagine activating 5-HT_{2B}R and 5-HT_{2C}R present in the gastric smooth muscle and brain. Administering these receptor antagonists to cisplatin-treated rats simultaneously restores reduced food intake and peripheral acylated ghrelin (11).

Acute stress increases the secretion of adrenaline and activates various receptors. Clonidine, an adrenergic α2 receptor agonist, also lowered peripheral acylated ghrelin concentration. Conversely, the addition of noradrenaline or adrenaline to the ghrelinoma cell lines stimulated ghrelin secretion, and this effect was blocked by atenolol (31), a selective β1-adrenergic antagonist. Systemic administration of isoproterenol and denopamine restores reduced blood acylated ghrelin levels in stress models. In a stress-like model with intracerebroventricular (ICV) urocortin injection, the administration of adrenergic α-receptor antagonist phentolamine and the α2 receptor antagonist yohimbine significantly improved the decreased acylated levels (32). The effect disappeared when administered along with a ghrelin receptor antagonist. This means that adrenergic α2

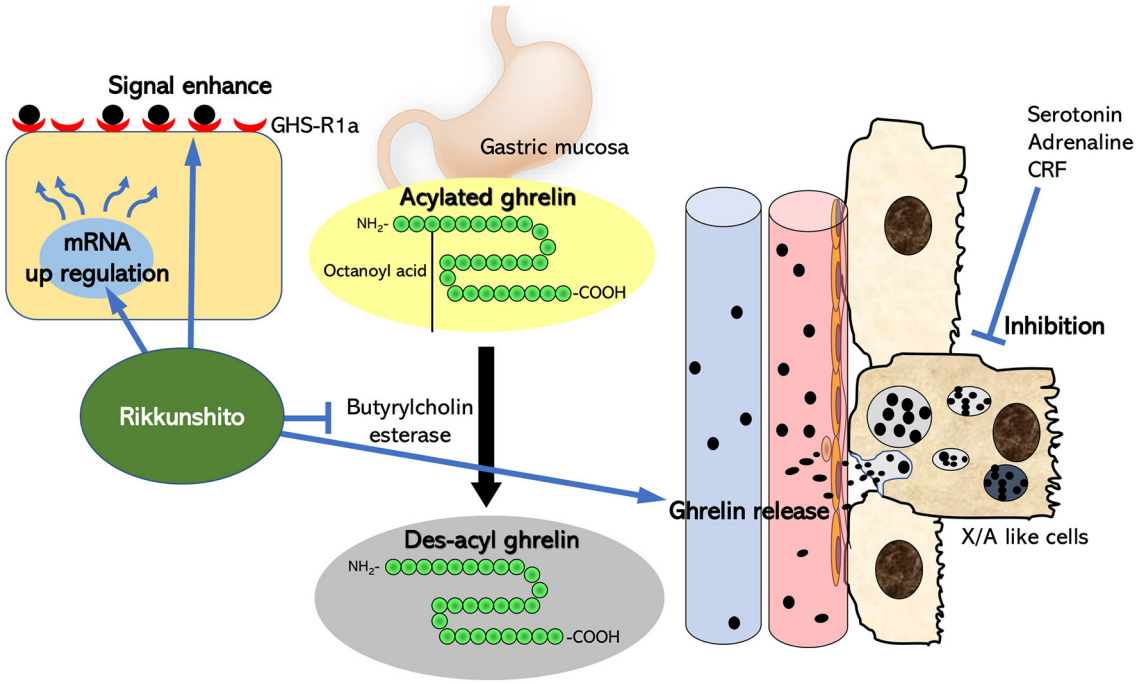

FIGURE 1 | Summary of the mechanism of action of rikkunshito as a ghrelin enhancer. Ghrelin is produced from X/A-like cells near the gastric mucosa, and the ghrelin receptors at the ends of the vagus nerve in the surrounding tissues are stimulated. Most of it migrates to blood vessels and is carried to the brain. Serotonin, adrenaline, and CRF negatively regulate ghrelin secretion in the periphery. Rikkunshito suppresses the decrease in ghrelin that occurs in diseases by antagonistically acting on these factors with receptors in the periphery or central nervous system. Furthermore, rikkunshito enhances the binding of ghrelin to the ghrelin receptor and also acts on the ghrelin signal transduction system itself to enhance the ghrelin signal.

receptor stimulation negatively regulated feeding through a decrease in ghrelin receptor activation. ICV administration of the corticotropin-releasing factor (CRF), a trigger factor for the HPA axis during stress, also reduces peripheral blood acylated ghrelin levels and food intake (29). Thus, abnormal acylated ghrelin secretion may be noticed in diseases in which these factors are closely related to pathology.

Promotion of Ghrelin Secretion by Rikkunshito

Normal Animals and Healthy Volunteers

A few reports examine the effects of the administration of rikkunshito on normal mice to increase appetite and acylated ghrelin production. Matsumura et al. measured blood acylated ghrelin concentration and gastric gene expression at 0.7–1.4% drinking water administration of rikkunshito to normal mice and showed a significant increase in the rikkunshito group compared with the saline-treated group (33). However, in our preliminary study, the amount of acylated ghrelin in the blood tended to increase 180 min after the administration of rikkunshito 1 g/kg by gavage (distilled water administration group, 55.7 ± 9.2 fmol/mL, vs. rikkunshito administration group, 72.3 ± 5.9 fmol/mL), and des-acyl concentration decreased (distilled water administration group, 729.6 ± 74.1 fmol/mL, vs. rikkunshito administration group, 407.1 ± 30 fmol/mL). It is considered that the difference in this result depends on the difference in administration period and administration method. Additionally, Matsumura et al. demonstrated that administration of rikkunshito 7.5 g/day to 21 healthy volunteers for 2 weeks significantly increased blood acylated ghrelin levels compared to that before administration (33). It is considered that the maintenance of physiological homeostasis related to the secretion of appetite-related hormones is strictly controlled. Therefore, the effect of a single dose of rikkunshito on healthy subjects may be limited. To affect normal ghrelin secretion function, rikkunshito may require continuous administration.

Side Effects of Chemotherapy and Cancer Cachexia

Animal Model

Table 1 summarizes the major indexes and ghrelin-promoting effects of rikkunshito on anticancer drug administration, organ removal, and cancer cachexia. Administering cisplatin to rodents results in reduced food intake. Peritoneal administration of cisplatin at 2 mg/kg significantly reduces rat feeding under fasting and free fed conditions up to 24 h. Gavage oral administration at a dose of 1 g/kg of rikkunshito was observed to significantly suppress the reduction in food intake due to cisplatin administration compared to saline-administered rats (11–13, 34, 35). Intraperitoneal administration of cisplatin 2 mg/kg decreases food intake and decreases blood acylated ghrelin levels and hypothalamic acylated ghrelin release 2 h

after administration. Rikkunshito has been proven to abolish these declines (11, 12, 35). Similarly, rikkunshito suppressed the decrease in food intake in rats given intraperitoneally at a higher dose of cisplatin (6 mg/kg) (36). Thus, the effect of rikkunshito on ghrelin is very reproducible, and it has been proven that rikkunshito inhibits feeding reduction and an acylated ghrelin secretion-promoting effect in cisplatin-administered rats. Moreover, in cancer-bearing animals, experimental exposure to cancer cells induces cachexia, depending on the type of cancer and the duration of the experiment, resulting in a marked decrease in food intake and body weight. Additionally, a significant reduction in food intake and body weight is also observed in gastrectomized rats (37). An extreme decline in feeding leads to increased starvation and induction of signal abnormalities in peripheral appetite-promoting peptides, despite the lack of appetite in these animal models. Particularly, peripheral acylated ghrelin increases, and exogenous acylated ghrelin reactivity also decreases the so-called ghrelin resistance (13, 38). Rikkunshito administration to cancer cachexia rats is shown to improve the decrease in food intake and prolong life. However, rikkunshito did not further increase blood acylated ghrelin. Rikkunshito not only increases peripheral ghrelin but also stimulates ghrelin receptor signaling and stimulates feeding (13, 38). This mechanism of action of rikkunshito will be focused on in detail at the bottom.

Clinical Study

In gastric cancer patients (39–41) and uterine cervical or corpus cancer patients (42), the efficacy of rikkunshito for ghrelin concentration and gastrointestinal dysfunction, including feeding, was evaluated. Ohno et al. reported that rikkunshito at a dose of 7.5 g/day suppressed the increase in oral intake and the decrease in acylated ghrelin due to cisplatin administration from the start of the combined administration of S-1 and cisplatin to patients with gastric cancer (39). Similarly, Takiguchi et al. (40) observed an increase in the acylated ghrelin ratio to total ghrelin at 4 weeks after administration of rikkunshito and an improvement in the Dysfunction after Upper Gastrointestinal Surgery for Cancer (DAUGS) and visual analog scale. These results clearly suggest that rikkunshito clinically promoted ghrelin secretion and suppressed feeding-related decline. However, in patients with proximal gastrectomy, weight gain and increase in the Gastrointestinal Symptom Rating Scale (GSRS) were noticed after administering rikkunshito. Still, they did not affect ghrelin levels (41). These findings suggest that rikkunshito improves gastrointestinal symptoms in patients with gastrectomy, but its effect on ghrelin has various results. This may be mediated by the fact that the main production site of ghrelin is the stomach. The conditions for collecting blood samples and the conditions for measuring acylated ghrelin may differ at each facility. Ohnishi et al. (42) found that rikkunshito (7.5 g/day) administration for 2 weeks in patients with cervical cancer improved appetite up to 2–6 days after paclitaxel administration and delayed onset 24–120 h later, and it significantly suppressed nausea and vomiting. However, acylated ghrelin did not change even after administration of an anticancer drug, and no effect was found by rikkunshito. Moreover, it was reported that there was no effect on nausea and vomiting by chemotherapy for lung cancer patients in the group taking rikkunshito 7.5 g/day for 7 days at the same time (43) but for anorexia. The 14-day administration of rikkunshito (7.5 g/day) inhibited the decrease in plasma acylated ghrelin, and the rate of decrease in calorie intake was lower in rikkunshito than in the control course (18 vs. 25%, $P = 0.025$) (44). Another researcher reported that the median rate of reduction in food intake was significantly lower with rikkunshito than without it (2 vs. 30%; $P = 0.02$) (45). Median acylated ghrelin increased significantly from day 3 to day 8 in patients on both courses with and without rikkunshito (9.6–15.7 fmol/mL, $P < 0.0001$; control, 10.2–17.8 fmol/mL, $P = 0.0002$). The rate of median increase in plasma acylated ghrelin levels between days 3 and 8 tended to be higher in the rikkunshito than in the control course (68 vs. 48%, $P = 0.08$). For delayed gastric emptying after pancreaticoduodenectomy, the 21-day administration of rikkunshito showed a significantly upregulating in total ghrelin and acylated ghrelin levels compared to preoperative, but no obvious effect on delayed gastric emptying was observed (46). It is believed that the effectiveness of rikkunshito will become clearer in future large-scale studies.

Stress-Induced Loss of Appetite

Stress is closely associated with appetite and exhibits an entirely different phenotype depending on the quality and duration of stress. Acute stress and stress loads that have a strong impact may primarily suppress feeding (52). In addition, a combination with chronic and mild stress may increase appetite and alter food preferences (53, 54). Previously, it has been reported that abnormal dynamics in acylated ghrelin are observed with stress loading and mediate abnormality in appetite. This review describes the effect of rikkunshito on the stress-induced loss of appetite.

Animal Model

Blood adrenocorticotropic hormone (ACTH) and corticosterone on the HPA axis in the rodent model are used as indicators of the degree of an acute stress response. Previous studies have not investigated in detail whether rikkunshito inhibits the HPA axis. It was confirmed that the administration of rikkunshito to stress-loaded aged mice significantly decreased the increase in ACTH or corticosterone value (55). This indicates that rikkunshito may act in a suppressive manner on stress itself.

Yakabi et al. (32, 47) and Harada (49) demonstrated that the ICV administration of urocortin 1, which has a strong affinity for the CRF receptor, significantly reduces feeding behavior and abnormal movement of the upper gastrointestinal tract. The concentration of acylated ghrelin in the peripheral blood was significantly reduced, simultaneously, and the supplementation of acylated ghrelin to the urocortin-treated rat significantly improved this decrease in food intake. Urocortin-induced reduction of plasma ghrelin and food intake were restored by CRF2 receptor antagonist. Administration of rikkunshito to urocortin-treated rats significantly improved reduced food intake, abnormal gastrointestinal motility, and acylated ghrelin levels (32, 47, 49). The adrenergic α2 receptor was activated in urocortin-administered rats, and it was also found that

TABLE 1 | The effect of rikkunshito on the main evaluation and on ghrelin in each study.

Years	Basic research	Ghrelin	Index	References
2008	Cisplatin-treated rats	↑	Food intake↑	Takeda et al. (11)
2010	Cisplatin-treated rats	↑	Food intake↑	Yakabi et al. (12)
2011	Cancer cachexia rats	↑	Food intake, survival↑	Fujitsuka et al. (13)
2011	Cisplatin-treated rats	↑	Food intake↑	Sadakane et al. (35)
2013	Cisplatin-treated rats	-	Food intake↑	Yoshimura et al. (36)
2016	Gastrectomied rats	→	Food intake↑	Taguchi et al. (37)
2017	Gastric cancer rats	signal ↑	Food intake↑	Terawaki et al. (38)
Years	**Clinical research**	**Ghrelin**	**Index**	**References**
2011	Cisplatin-treated patients with gastric cancer	↗	Food consumption↑	Ohno et al. (39)
2011	Cancer cachexia patients	↑	Survival↑	Fujitsuka et al. (13)
2013	Gastric cancer	↑	Food consumption, DAUGS score↑	Takiguchi et al. (40)
2013	Gastrectomy	→	Body weight, GSRS↑	Gunji et al. (41)
2017	Uterine cervical or corpus cancer patients	→	CINV↓	Ohnishi et al. (42)
2017	Lung cancer patients with chemotherapy	-	CINV →	Harada et al. (43)
2019	Cisplatin-treated patients with esophageal cancer	↑	Food consumption↑	Hamai et al. (45)
2020	Cisplatin-treated patients with lung cancer	↑	Food consumption↑	Yoshiya et al. (44)
2020	Pancreaticoduodenectomy	→	Delayed gastric emptying →	Yamaguchi et al. (46)
Related to stress				
Years	**Basic research**	**Ghrelin**	**Index**	**References**
2011	Urocortin 1-treated rats	↑	Food intake ↑	Yakabi et al. (47)
2011	Novelty stressed mice	↑	Food intake ↑	Saegusa et al. (29)
2013	Novelty stressed mice	↑	Food intake ↑	Yamada et al. (30)
2014	Urocortin 1-treated rats	↑	Food intake ↑	Yakabi et al. (32)
2014	Acute restrained stressed mice	signal ↑	Gastric motility↑	Nahata et al. (48)
2015	Urocortin 1-treated rats	↑	Gastric emptying↑	Harada et al. (49)
2020	Novelty stressed mice	-	Food intake ↑	Yamada et al. (50)
Years	**Clinical research**	**Ghrelin**	**Index**	**References**
2011	Esophageal cancer patients with chemotherapy	-	Nausea ↓	Seike et al. (51)
2014	Non-erosive reflux disease	-	MCS score↑	Tominaga et al. (2)

CINV, chemotherapy-induced nausea and vomiting; DAUGS, Dysfunction After Upper Gastrointestinal Surgery for Cancer; GSRS, Gastrointestinal Symptom Rating Scale; MCS, mental component summary.

rikkunshito contained a component with an antagonistic effect on the receptor (32, 49). However, the administration of an adrenergic β1 receptor agonist also improves urocortin-induced ghrelin lowering, but it is not confirmed whether or not rikkunshito contains a β1 agonist-like ingredients component.

Rodents are often bred and managed with 3–5 animals depending on the cage size. Acute stress can be induced by acclimating to this environment for about a week and then transferring to a completely new cage and bedding (28–30, 50). Novel environmental changes can cause mild and transient corticosterone increase and decreased feeding in mice. Additionally, the concentration of acylated ghrelin in the blood decreases at the same time as stress loading (28–30). Rikkunshito significantly reduced the decrease in food intake and reduction in blood acylated ghrelin concentration due to this novel environmental change stress (28–30). Since the decreased food intake and ghrelin secretion in this model are partially canceled by the administration of 5-HT_{2B}R or 5-HT_{2C}R antagonists, serotonin is involved in the decreased food intake in the brain and digestive organs (28–30). ICV administration of CRF1R antagonists mediates reductions in food intake and plasma acylated ghrelin secretion (29), suggesting that intracerebral CRF1R activation is the trigger for the onset of this model. Restraint stress is known as classical physical and mental stress. Restraint stress in mice causes dysfunction of the upper gastrointestinal tract motility and a decrease in acylated/des-acyl ghrelin ratio (48). Administration of rikkunshito to stress mice significantly improved gastric motor function abnormalities such as delayed gastric emptying and gastric motility index. It is speculated that these action by rikkunshito may have canceled the decreased feeding and ghrelin secretion deficiency due to stress.

Clinical Study

There are few clinical evidences focusing on the efficacy of rikkunshito on stress and mental illness. Tominaga et al. found that taking rikkunshito (7.5 g/day) for 8 weeks in patients with proton pump inhibitor-resistant non-erosive reflux disease (NERD) represents the mental quality of life in patients with a low body mass index. It proved that the mental component summary (MCS) scores of the SF-8 was significantly improved

by rikkunshito (2). Additionally, administration of docetaxel/5-FU/CDDP in patients with advanced esophageal cancer for 2 weeks with rikkunshito (7.5 g/day) significantly improved nausea, as well as sleep, mood, volition, daily living activity, and anxiety and greatly improved quality of life scores, including the feeling of anxiety (51). Although the results of these clinical trials do not show the direct anti-stress effect of rikkunshito, it led to the implementation of larger clinical trials and it is expected to find the usefulness of rikkunshito for gastrointestinal disorders and neuropsychiatric parameters due to stress loading.

Mechanism of Action of Rikkunshito

Antagonists on Serotonin, CRF, and Adrenergic Receptors

Ghrelin release in the stomach and hypothalamus is negatively regulated by 5-HT_{2B}R and $_{2C}$R activation (11). Heptamethoxyflavone, hesperetin, nobiletin, tangeretin, and isoliquiritigenin, which are components of rikkunshito, have an antagonistic activity against 5-HT_{2B}R and $_{2C}$R *in vitro*. Additionally, these components, when administered alone *in vivo*, suppressed a decrease in blood ghrelin levels (11). Isoliquiritigenin has been confirmed to transfer to the brain after the administration of rikkunshito and may mediate the decrease in ghrelin secretion due to antagonism of 5-HT_{2C}R localized in the central nervous system (56).

It is suggested that stress-related hypophagia involves abnormalities in ghrelin kinetics mediated by CRF1 and adrenergic receptors, and stimulation of CRF1 and α receptors negatively regulates ghrelin secretion. Nobiletin and isoliquiritigenin antagonize the CRF1 receptor at IC50 values of 0.36 and 0.67 μmol/L, respectively (56). In addition, glycycoumarin has an IC50 value of 5–39 μmol/L for all subtypes ($_{A,B,C}$) of the α_2-adrenergic receptor (AR). The IC50 value of 6-shogaol, which is a component of *Zingiberis rhizoma*, is 25 μmol/L for α_{2A}-AR, 8-shogaol is 5–6 μmol/L for $\alpha_{2A,}$ α_{2C}-AR, and 10-gingerol is has an IC50 value of 5–31 μmol/L for $\alpha_{2A,}$ $\alpha_{2B,}$ α_{2C}-AR (32).

Ghrelin Receptor Stimulating Effect

Previous findings have shown that rikkunshito promotes acylated ghrelin secretion, but it does not increase it beyond physiological secretion. Therefore, it was questioned whether this degree of action could improve the reduced food intake. Rikkunshito enhanced the binding to ghrelin in cells expressing GHS-R1a *in vitro* and further significantly increased [Ca^{2+}] influx in the area under the curve by ghrelin. At the same time, it was discovered that the action was caused by the ingredients of rikkunshito, i.e., atractylodin (13). This finding means a new mechanism in which rikkunshito not only increases the blood acylated ghrelin concentration but also enhances the reactivity of acylated ghrelin with GHS-R1a, thereby increasing the ghrelin signal.

Another stimulating effect of the ghrelin signal has been studied and proposed. Rikkunshito mediates the production of cAMP through the inhibition of phosphodiesterase III against the adenylate cyclase-cAMP-PKA system involved in the ghrelin signal inhibitory effect of leptin via the PI3K-PDE pathway and the activation of ghrelin receptors (57). Further detailed research is required to determine how much this effect of rikkunshito affects the ghrelin signal.

NEW POSSIBILITIES

Aging, Gender Difference, and Healthy Life Expectancy

Maintaining the diet of the elderly is an important issue as a strategy to prevent sarcopenia in the global aging society. Additionally, aged people tend to lose their appetite due to changes in taste and decreased calorie consumption due to lack of exercise. Additionally, aged people often have many diseases, and maintaining the so-called healthy life expectancy and maintaining good quality of life are of utmost importance. In rodent studies, a flattening of ghrelin levels was observed in aged mice, with clearly reduced fasting ghrelin secretion compared to younger mice and, conversely, increased ghrelin levels during satiety (57). Additionally, changes in feeding behavior were also observed. Although the meal amount and meal size of the aged mice did not change compared with those of the younger mice, the number of activities at night and the food bout size were small, and the bout numbers were many. Thus, it eats little by little over time (58). Rikkunshito restores reduced feeding in aged mice without affecting acylated ghrelin levels (57).

So far, as a clinical evaluation, the impression that rikkunshito may be more effective for females than for males has been conveyed. However, there has been no evidence of gender differences in the effects of rikkunshito. Yamada et al. (50) studied and compared the effects of rikkunshito on decreased food intake in male and female mice exposed to psychological stress. Because of comparing female and male mice for events after stress loading, there was no gender difference in HPA axis activation, but food intake decreased more continuously in female mice. Female mice have a delayed increase in ghrelin rather than males and reduced responsiveness to exogenous ghrelin. In stress-loaded mice, rikkunshito showed an obvious effect of improving feeding in female mice. The cause of anorexia in female mice is thought to be ghrelin signal transduction failure in NTS, and rikkunshito was found to improve this signal disorder. Rikkunshito was more effective in women or the elderly (65 years and older) for FSSG in NERD patients (2). However, there is still no direct evidence that the action of rikkunshito as a ghrelin enhancer mediates gender differences and efficacy in the elderly. Further elucidation of the mechanism is required in the future.

It is known that calorie restriction activates various factors, including sirtuin (SIRT), and is involved in maintaining the functional decline of different tissues with aging (59). Particularly, activation of SIRT in the hypothalamus is triggered by calorie restriction and is associated with the maintenance of energy balance homeostasis (60–62). Ghrelin may increase the orexigenic signal and may cause the same condition as calorie restriction. For example, ghrelin activates adenosine monophosphate-activated protein kinase (AMPK) (63), and SIRT is also activated by AMPK (64). Therefore, it is easy to hypothesize that rikkunshito, which promotes ghrelin secretion

and receptor activation, may cause SIRT activation. Fujitsuka et al. evaluated the effect of rikkunshito on healthy life expectancy using various aging-promoting mice (65). Rikkunshito induced activation of SIRT1 in the hypothalamus *in vivo*. It was also discovered that the effect was not expressed in ghrelin KO mice. Furthermore, administration of rikkunshito to ICR mice as aging-promoting models such as Klotho-deficient mice, SAMP8 mice, and normal-aged mice showed prolonged survival. Again, focal atrophy of myocardial fiber and pericarditis was significantly decreased in mice treated with rikkunshito. This study is a basic study using mice, but we hope that a clinical review will be conducted in the future and that rikkunshito will prove the possibility of improving the QOL of the elderly.

SUMMARY

The promoted ghrelin secretion and ghrelin signal promotion exerted by rikkunshito may play an important role for effectiveness for (i) anorexia, nausea and vomiting due to chemotherapy, (ii) severe loss of appetite and weight loss due to cancer cachexia and stress-induced hypophagia, and (iii) hypophagia in the elderly. Evidence accumulated over the last few years shows that rikkunshito is particularly effective against the loss of appetite and gastrointestinal disorders caused by chemotherapy. Additionally, as result of basic research, rikkunshito may be involved in eating disorders and in extending healthy life expectancy in the elderly. However, based on the valuable basic research obtained so far, the evaluation of large-scale clinical trials may lead to further evidence of the usefulness of rikkunshito for the benefit of patients, moreover even Japanese Kampo medicine.

AUTHOR CONTRIBUTIONS

CY and TH study design, data collection and analysis, and drafting of the manuscript. TH, SO, and HT study supervision. All authors contributed to the article and approved the submitted version.

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REVIEW
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A Review on the Mechanism and Application of Keishibukuryogan

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The concept of "blood stasis" – called yū xiě in Chinese, Oketsu in Japanese – is one of the unique pathophysiology of traditional medicine that originated in China and inherited in Korea and Japan. This concept is related to the multiple aspects of hemodynamic disorders brought on by quantitative and qualitative changes. It theorizes that the quantitative changes of "blood stasis" are related to peripheral circulatory insufficiency. When chronic qualitative changes of "blood stasis" produce stagnant blood that turns into a pathological product, it could cause inflammation and lead to organic changes. Trauma induced hematomas, that are considered to be a quantitative change of blood, are also a form of blood stasis. The basic medicine research on Keishibukuryogan (KBG)–a Japanese name in Traditional Japanese Medicine (Kampo) for one of the most common anti- "blood stasis" prescriptions, also known as gui-zhi-fu-ling-wan (GFW) in Chinese in Traditional Chinese Medicine (TCM)–indicated that the initiation of quantitative changes was closely related to loss of redox balances on endothelial function induced by oxidative stress. The following qualitative changes were related to coagulopathy, hyper viscosity; anti-platelet aggregation, lipid metabolism; a regulation of systemic leptin level and/or lipid metabolism, inflammatory factor; cyclooxygenase-1,2 (COX-1, 2), interleukin-6, 8 tumor necrosis factor-α, macrophage infiltration, hyperplasia, tissue fibrosis and sclerosis caused by transforming growth factor-β1 and fibronectin, the dysfunction of regulated cell deaths, such as, apoptosis, autophagy, ferroptosis and ovarian hormone imbalance. Clinically, KBG was often used for diseases related to Obstetrics and Gynecology, Endocrine Metabolism, Rheumatology and Dermatology. In this review, we give an overview of the mechanism and its current clinical application of KBG through a summary of the basic and clinical research and discuss future perspective.

Keywords: Kampo, blood stasis, Oketsu, TCM, traditional medicine, Keishibukuryogan, gui-zhi-fu-ling-wan

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INTRODUCTION

The concept of "blood stasis" -called yu xiě in Chinese, Oketsu in Japanese–is one of the unique pathophysiology of traditional medicine that originated in China and inherited in Korea and Japan. This concept is related to the multiple aspects of hemodynamic disorders brought on by quantitative and qualitative changes. It theorizes that the quantitative changes in the blood are related to peripheral circulatory insufficiency. According to 211 studies about "blood stasis" in Korean Traditional Medicine as well, which were 19 reviews, 52 clinical studies and 140 preclinical studies, "stagnant blood within the body" was the most frequently mentioned phrase of the traditional concept of blood stasis, followed by "disorder of blood circulation," "pathological

product," "the blood lost its physiological function," "extravasated blood," "blood congested in viscera and tissue," "foul blood," "blood congested in a blood vessel," "organ dysfunction" and "stagnation of blood flow in local parts." Among these, the quantitative concepts of peripheral circulation disorders are suggested by "disorder of blood circulation," "blood congested in viscera and tissue," "blood congested in a blood vessel" and "stagnation of blood flow in local parts." "Pathological product," "the blood lost its physiological function," "foul blood" suggested qualitative changes due to chronic "blood stasis." In the preclinical studies in Korea, coagulopathy was studied most frequently, followed by hyper viscosity, hyperlipidemia, inflammation, neoplasm, ischemic brain injury, and atherosclerosis. In the clinical studies, traumatic injury was the most frequently studied disease/condition, followed by genitourinary and cerebrovascular disease (1). In this review, we give an overview of the mechanism and its current clinical application of KBG–a Japanese name in Traditional Japanese Medicine (Kampo) for one of the most common anti- "blood stasis" prescriptions, also known as gui-zhi-fu-ling-wan (GFW) in Chinese in Traditional Chinese Medicine (TCM)-through a summary of the basic and clinical research and discuss future perspective.

BASIC RESEARCH

The effects of KBG in basic research are mainly about antioxidant, Nitric Oxide (NO) production, vasodilatory effects, and suppression of inflammatory cytokine production, all of which are closely related. The concepts of qualitative and quantitative changes of "blood stasis" overlapped. Peripheral circulatory insufficiency, a quantitative change, is likely to cause micro-inflammation due to active oxygen and inflammatory cytokines. Chronic qualitative changes altered lipid metabolism and dynamics of female hormones and produced "stagnant blood" that turns into a pathological product, which could cause inflammation and lead to organic changes, such as hyperplasia, fibrosis and sclerosis. These results are summarized on **Table 1**.

The Vasodilatory Effect by the Increasing NO Production

Tomita, et al. visualized the immediate vasodilatory effect of KBG to investigate "peripheral circulatory insufficiency" -one pathological aspect of "blood stasis." KBG induced significant vasodilation and improved blood velocity in arterioles of murine subcutaneous vessels detected by live imaging technics. This vasodilation peaked 60 min after administration and persisted for 90 min (2). The visualized image of rat mesenteric arterioles after KBG administration was evaluated using erythrocyte congestion, broadening the cell free layers as the pathology of the "blood stasis." This study revealed an increase of nitric oxide, an endothelium-derived relaxing factor (EDRF), in the arterial endothelium of rat mesenteric arteries, especially at bifurcations, following KBG administration by live imaging (2). They concluded that the vasodilatory effect of KGB is due to the increasing NO production in the vascular endothelial cells.

Protective Effect Against NO-Induced Neurotoxicity and Inflammation

Other than the vasodilatory effect of Nitric Oxide by eNOS, NO could cause cytotoxicity. Shimada, et al. reported that KBG was effective against NO-induced neurotoxicity caused

TABLE 1 | The effects of KBG in basic medicine research.

Effects	Related substances	Related pathways	Targets	Reference
vasodilation	NO, EDRF		endothelial cell	(2, 3)
anti-platelet aggregation			platelet	(4)
antioxidant	lipid oxidation, superoxide dismutase, xanthine oxidase, VCAM-1		endothelial cell, erythrocyte	(3, 5–13)
anti-inflammation	NO, MIF, IL-1β, 6, 8, PGE-2, TNF-α, COX-1,2,	TNF, NF-kappa B		(8, 9, 14, 15)
macrophage infiltration	MCP-1, VCAM-1, osteopontin			(8)
anti-fibrotic (accumulation of ECM)	TGF-β1, fibronectin, fibroblast proliferation, TNF-α, MIP-2, IL-6, collagen production,	VEGF signaling, Toll-like receptor signaling	kidney, liver	(8, 9, 12, 16, 17)
lipid metabolism	adipocytokine (leptin, TNF-alpha), adiponectin		adipocyte, liver, epididymis	(18, 19)
against atherosclerosis	lipid peroxidase, oxidative LDL modification			(10, 11)
regulated cell deaths	ferroptosis,	p62-Keap 1-NRF2 p	endometrial hyperplasia	(13)
	autophagy,	PI3K/AKT/mTOR	uterus	(20)
hot flash	CGRP			(13, 21, 22)

Related substances, NO (Nitric Oxide), EDRF (endothelium-derived relaxing factor), VCAM (vascular cell adhesion molecule)-1, MIF (Macrophage migration inhibitory factor), IL (Interleukin)-1β, 6, 8, PGE (Prostaglandin)-2, TNF (Tumor Necrosis Factor)-α, COX (cyclooxygenase)-1,2, MCP (monocyte chemoattractant protein)-1, TGF (transforming growth factor)-β1, MIP-2 (macrophage inflammatory protein), LDL (Low Density Lipoprotein), CGRP (calcitonin gene-related peptide).

Related pathways: TNF (Tumor Necrosis Factor), NF-kappa B (nuclear factor-kappa B), VEGF (vascular endothelial growth factor), PI3K/AKT/mTOR (phosphatidylinositol 3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR).

by activation of neuronal NO synthase (nNOS). KBG had protective effect against NO-mediated neuronal death in cultured cerebellar granule cells and its effect is derived from Cinnamomi Cortex, Paeoniae Radix and Moutan Cortex (14). The association between the inhibitory effect of KBG on inflammatory cytokines and NO has been reported. Yoshihisa et al. evaluated the role of KBG in inhibiting the inflammatory cytokines using human dermal microvessel endothelial cells. KBG as well as paeoniflorin treatment significantly suppressed the mRNA levels of migration inhibitory factor (MIF), IL-6, 8 and tumor necrosing factor-α in LPS stimulated cultured human dermal microvessel endothelial cells. ELISA also showed KBG as well as paeoniflorin suppressed the production of these cytokines. In addition, KBG and paeoniflorin suppressed the expression of cyclooxygenase-2 and inducible nitric oxide synthase(iNOS) in these cells (15). This study suggested that KBG showed inhibitory effect on inflammatory cytokines by suppression against iNOS in skin endothelial cells and its mechanism of action.

Protective Effect on the Endothelial Function Against Oxidative Stress

Diacron-reactive oxygen metabolites (d-ROMs) is a simple test for measuring oxidative stress in plasma. The antioxidant activity of Kampo prescription using d-ROMs had also been reported. Keishibukuryogan-ka-yokuinin, which is KBG plus one herbal medicine, yokuinin, which is coix seeds (*Coix lacryma-jobi var. ma-yuen*), decreased level of the d-ROMs in plasma. Reactive oxygen species (ROS)-scavenging and lipid hydroperoxide generation assays revealed that gallic acid, 3-O-methylgallic acid, (+)-catechin, and lariciresinol possess strong antioxidant activities (5). Nozaki, et al. reported the KBG's protective effect of vascular function by anti-oxidative effect. This study reported that KBG protected the endothelial function of Adjuvant-induced arthritis (AIA) rats mainly by its anti-oxidative effect. KBG improved endothelium-dependent relaxation by acetylcholine in the AIA and decreased the contractions by xanthine oxidase, plasma level of lipid oxidase and restricted increase of the expression of endothelial NO synthase, inducible NO synthase (iNOS) and VCAM-1 of thoracic aorta (3). Other than KBG, many herbal prescriptions are known to possess beneficial effects against oxidative stress (6).

Protective Effect on RBC Against Oxidative Stress

Sekiya et al. reported that KBG provided a protective effect against the fragility of erythrocyte cell membranes caused by active oxygen (7). This study was an attempt to explain the concept of blood stasis by the relationship between active oxygen and red blood cells in the blood. Since blood stasis was also defined as "red blood's stasis" in medical classics in China, it is possible that the pathophysiology of red blood cells and blood stasis is related. Sekiya, et al. reported that KBG provided strong protection for RBC membranes against haemolysis induced by 2,2-azo-bis (2-amidinopropane) dihydrochloride, an azo free radical initiator. Inhibitory effect was dose dependent at concentration of 100–1,000 microg/ml. Furthermore, ingestion of 200 mg of KBG was associated with a significant decrease in susceptibility of RBC to haemolysis in rats (7). Another study of the pathophysiology of "blood stasis" about RBC, though in humans, reported that not only erythrocyte aggregability but also deformability was related to the "blood stasis" (23).

Inhibitory Effect on Platelet Aggregation

The relationship between coagulopathy, hyperviscosity and "blood stasis" was studied by the effect of KBG on anti-platelet aggregation. The platelet aggregation was measured by pressure rate and the platelet aggregatory threshold index (PATI) values on collagen-induced platelet aggregation of guinea pig whole blood. Significant difference was observed in 1,000 μg/mL-KGB group ($P < 0.0001$) compared to control group. KBG inducement suppressed the collagen-induced whole blood pressure rate increase and increased the PATI value. Focusing on the herbal ingredients of KBG, paeonol, a representative component of Moutan cortex, and aspirin which is known to have platelet aggregation-inhibitory activity (COX-1 inhibitor) also showed similar effects. Based on these results, they suggested that the platelet aggregation-inhibiting activity of the constituent crude drug Moutan cortex and Cinnamomi cortex is involved in the improved effects of KBG on impaired microcirculation and that paeonol plays a role in these effects (4).

Anti-inflammatory Effect by Decreasing COXs' Expression

Zhang et al. evaluated the anti-inflammatory functions of KBG, as well as its major ingredients, in human umbilical vein endothelial cells (HUVECs). The application of KBG significantly downregulated the mRNA expressions of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) mRNAs in dose-dependent manner. Nine major components of KBG were tested in the inflammatory system, and three compounds-paeoniflorin, benzoylpaeoniflorin, and amygdalin-exhibited robust activation in HUVECs. The combination of paeoniflorin, benzoylpaeoniflorin, and amygdalin showed over 80% of the anti-inflammatory activation (8).

Another study reported that compared with LPS (Lipopolysaccharide) treated group, KBG and its active complex dose-dependently reduced the releasing of IL-1β, TNF-α and Prostaglandin E2 (PGE2) induced by LPS in RAW264. Seven cells. Moreover, the expression of IL-1β and microsomal prostaglandin E2 synthase-1 (mPGES-1) was decreased after KBG and its active complex treatment, which might contribute to the inhibitory effect of KBG in the releasing of IL-1β, TNF-α and PGE2 (9).

Nephroprotective Effect

There are studies that report its efficacy as prevention of progression of chronic renal failure. Based on the effect of KBG against kidney of 5/6 nephrectomized rats, a well-characterized model of chronic renal failure (CRF), Nakagawa et al. reported that KBG exerts beneficial effects that results in slowing the progression of CRF (16). This study investigated the following effects of KBG against I) macrophage infiltration, II) accumulation of extracellular matrix (ECM), and III) oxidative

stress, all of which are considered to be the pathogenesis of the development of CRF.

Inhibitory Effect on Macrophage Infiltration

Macrophage infiltration is evaluated by osteopontin, MCP-1 and VCAM-1 mRNA levels. The administration of KBG significantly suppressed osteopontin, while it showed a tendency to decrease MCP-1 and VCAM-1 mRNA levels without statistical significance. Osteopontin, potent chemotactic and adhesion molecule for monocyte/macrophage, has been shown to have strong association with focal macrophage infiltration in a number of experimental models of renal injury, suggesting a pathologic role in progressive renal injury. They concluded that these results suggest that KBG inhibits macrophage infiltration by suppression of mRNA levels related to macrophage infiltration (16).

Inhibitory Effect of Accumulation of Extracellular Matrix

Accumulation of ECM proteins, such as fibronectin, type IV collagen and laminin, conspicuous finding accompanying the progression of renal failure, is one of the pathogeneses of progressive renal failure. TGF-beta1 has been implicated as playing central role in the regulation of the over- deposition of ECM proteins. KBG treatment significantly suppressed mRNA levels of TGF-beta1 and fibronectin, suggesting that KBG exerts beneficial effects on the kidney by inhibiting ECM protein accumulation accompanying the progression of CRF induced by TGF-beta (16).

Inhibitory Effect Against Oxidative Stress

KBG administration showed significant reduction in serum urea nitrogen and urinary protein, not creatinine, compared with the control group. Oxidative stress is widely recognized to involve the pathogenesis of CRF. They speculated that KBG improved oxidative condition and microcirculation in the kidney of CRF, and these effects may contribute, at least in part, to the attenuation of serum urea nitrogen and urinary protein excretion, not serum creatinine. They reported in another article that KBG decreased lipid peroxidation and elevated superoxide dismutase activity in the kidney (18, 24). Their speculation about significant reduction in serum urea nitrogen and urinary protein by KBG was based on their previous research about KBG's oxidative effect. The effect of KBG on serum creatinine was unchanged (16) and reduced (24). Nakagawa et al. reported that oral administration of KBG in spontaneous diabetic WBN/Kob rats significantly attenuated urinary protein excretion and serum creatinine level. KBG also reduced fibronectin and TGFβ1 protein expression of the renal cortex. Furthermore, lipid peroxidation levels in both kidney and liver were significantly lower than those of untreated control WBN/Kob rats. Urinary expression of 8-hydro-deoxyguanosine, an oxidative stress marker, was suppressed by KBG treatment. These results suggest that KBG reduces oxidative stress by hyperglycemia, and it protects renal function and suppresses fibronectin deposition induced by TGFβ1 production in WBN/Kob rats (24)."

The Effect of KBG on Renal Transporter as Nephroprotective Agents

Lee S.H. et al. hypothesized that KBG may modulate the renal transporter function, URAT1, OAT1 and OAT3, which are responsible for the renal reabsorption of uric acid and mediate the renal uptake of organic anions, drugs, and metabolic toxins, as the primary contributors to the drug-induced nephrotoxicity (19). They reported that KGB inhibited the substrate uptake activities of renal transporter, the urate transporter 1 (URAT 1), the organic anion transporters (OAT1 and OAT 3) in Xenopus oocyte and HEK 293 human kidney embryonic cells, suggesting their mechanism of action as nephroprotective agents. OAT-organic anion transporter-membrane proteins that mediate the translocation of diverse compounds across biological membranes, occupy the largest portions in the regulation of kidney physiological processes. About the relationship between KBG with renal transporters, further research is expected on the specific renal protective effect of KBG.

Regulation of Systemic Adipocytokines Level and/or Lipid Metabolism

The following reports suggested that KBG could improve obese status through a regulation of systemic leptin level and/or lipid metabolism. Adipocyte hypertrophy and adipocytokines, such as leptin and TNF-α, are considered to be key pathological contributors to insulin resistance.

Gao et al. reported that KBG treatment significantly decreased the serum level of leptin and liver triglyceride (TG) level in the diet-induced obesity mouse (10). In addition, a lower fat deposition in liver and a smaller size of adipocytes in white adipose tissue were observed in the diet-induced obesity mouse treated with KBG. They found downregulation of genes involved in lipid metabolism in the KBG-treated liver, along with decreased liver TG and cholesterol level (10).

Nakagawa et al. reported that KBG significantly lowered serum total cholesterol and triglyceride levels, and the hepatic total cholesterol, and reduced serum leptin level, not the serum adiponectin level in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes. They also showed significant effects of KBG on epididymal adipose tissue by decreasing the size of fat cells and on skeletal muscle by reducing TNF-α protein content, a crucial factor responsible for insulin resistance in obese and diabetic subjects (11). They suggested that KBG exerted a hypolipidemic effect in the diabetic body and this effect contributed to amelioration of the insensitivity of peripheral tissues to insulin and glucose disposal.

Inhibiting the Progression of Atherosclerosis in Hypercholesterolemia by Antioxidant Effect

Sekiya et al. reported in their study of cholesterol-fed rabbits, that of the control, the KBG and vitamin E groups, the platelet counts at the end of the study were significantly lower than those determined prior to study and the progression of visible plaque was inhibited in KBG groups compared to the control ($P < 0.01$). The serum lipid peroxidase was significantly lower in

KBG and vitamin E groups, compared with control group ($P < 0.05$) and urinary 8OHdG-an oxidative marker-was significantly lower in KBG group compared with vitamin E group ($P < 0.05$). These results in cholesterol-fed rabbits suggested that KBG prevented the progression of atheromatous plaque by creating a sounder antioxidant defense system than vitamin E as lipid soluble antioxidant (12). They previously reported that aortic surface involvement of control rabbits were greater than that of KGB treated rabbits statistically ($P < 0.01$), in both LDL and beta-VLDL, and lipid peroxide formation in KBG treated rabbits were less than that in control rabbits statistically ($P<0.01$). KBG suppressed the *in vitro* lipid peroxide formation dose-dependently compared with control. KBG administered rabbits showed the suppression of serum lipid peroxidase formation compared with rabbits before its administration ($P < 0.05$). Based on this result, they concluded that KBG prevents the progression of atherosclerosis in cholesterol-fed rabbits *in vivo* by limiting oxidative LDL modification (25).

Inhibitory Effect on Fibrosis Against Oxidative Stress

Fujimoto et al. investigated that KBG could be a candidate to prevent the progression of non-alcoholic fatty liver disease (NAFLD) to steatohepatitis (NASH) in standard rabbits (26). The result suggested that KBG was superior to vitamin E and pioglitazone in the reduction of the liver total cholesterol ($P < 0.01$) and lipid peroxidase ($P < 0.05$), urinary 8-hydroxy-2' deoxyguanosine ($P < 0.05$), hepatic α-smooth muscle actin (α-SMA) positive areas ($P < 0.05$) and activated stella cells ($P < 0.05$). A-SMA is a specific marker for smooth muscle cell differentiation, which is related to the process of hepatic fibrosis, and is a reliable marker of hepatic stella cell activation which precedes fibrosis tissue deposition (17, 27, 28). They concluded that there was a statistically significant benefit of KBG, in particular, on a dietary model of NAFLD/NASH. Two-hit hypothesis is prevailing theory for the development of NAFLD. Oxidative stress is considered one of factors to cause "second hit." In this study, the process of organic disease, a chronic qualitative change caused by oxidative stress could be considered as one of the pathological conditions of "blood stasis."

Anti-fibrotic Effect on Systemic Sclerosis

There were studies related to "sclerosis" as a chronic qualitative change of "blood stasis." KBG could be regarded as a synergistic therapeutic option which takes pharmacological actions by affecting multiple signaling pathways and different molecules rather than a single pathway. System biologic approaches suggested that KBS could suppress the proliferation of fibroblasts and decrease the Th1 cytokines; TNF-α, MIP-2 and IL-6 (29). Another study investigated the effect of KBG on collagen production in scleroderma fibroblast culture. KBG significantly ($P < 0.05$) inhibited collagen product at each incubation time at 0, 6, 12 and 24 h, in a dose-dependent manner. A significant difference was demonstrated between the untreated and KBG-treated condition in each fibroblast line from three scleroderma patients. Sheng, et al. concluded that KBG significantly and selectively inhibited collagen synthesis in a dose-dependent manner, with a tendency of a stronger effect on scleroderma fibroblasts than control cells (30).

The Effect on Endometrial Hyperplasia by Triggering Ferroptosis

The term ferroptosis was coined in 2012 to describe an iron-dependent regulated form of cell death caused by the accumulation of lipid-based reactive oxygen species (31). It is morphologically, biochemically, and genetically distinct from apoptosis, necrosis, and autophagy (32). Recent studies have shown that ferroptosis is closely related to the pathophysiological processes of many diseases, such as tumors, nervous system diseases, ischemia-reperfusion injury, kidney injury, and blood diseases (33).

Zhang et al. investigated the effect of KBG on endometrial hyperplasia from the viewpoint of ferroptosis. According to their research, the degree of ferroptosis in endometrial tissue of patients was lower than in normal endometrial tissue. In addition, ferroptosis inducer imidazole ketone erastin (IKE) could improve endometrial hyperplasia in mice. Interestingly, KBG significantly alleviated endometrial hyperplasia through triggering ferroptosis. Furthermore, in estradiol-induced endometrial hyperplasia model, KBG inhibited p62-Keap1-NRF2 pathway which is the major regulator of cytoprotective responses to oxidative and electrophilic stress and is related to increasing cancer chemoresistance and enhancing tumor cell growth (13, 21). They concluded that KBG may attenuate estrogen-induced endometrial hyperplasia in mice through triggering ferroptosis *via* inhibiting p62-Keap1-NRF2 pathway (13).

Inhibitory Effects of Autophagy on Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a common reproductive and endocrinologic disorder and three main phenotype characteristics of this condition are hyperandrogenism, polycystic ovaries, and ovulatory dysfunction (22). This syndrome can also be associated with metabolic issues including obesity, insulin resistance (found in 60–80% of women with PCOS) (34). hyperinsulinemia, and type 2 diabetes mellitus (T2DM). The cause of PCOS remains largely unknown, but studies suggest an intrinsic ovarian abnormality such as granulosa cell survival and proliferation (20).

Liu et al. reported that KBG inhibited granulosa cell autophagy and promoted follicular development to attenuate ovulation disorder in PCOS-insulin resistance rats. They concluded that this result was associated with activation of the phosphatidylinositol 3-kinase (PI3K)/AKT/ mammalian target of rapamycin (mTOR) signaling pathway, which plays an important role in the regulation of cell survival, growth, and proliferation (20).

Das et al. reported that apoptosis is associated with the pathophysiology of PCOS (35). It is necessary to verify the effect of KBG on regulated cell deaths, such as apoptosis, necroptosis, and autophagy.

The Possible Effect on Initiation and Growth, Related to Inflammation, Fibrosis, Proliferation, and Angiogenesis of Uterine Fibroids

Uterine leiomyomas (ULs), also called uterine fibroid, arise due to transformation of the layer of smooth muscle cells of corpus uteri. Despite frequent occurrence of this disease, the molecular mechanisms behind the origin and development of leiomyomas are still relatively unknown (36). However, dysregulation of inflammatory processes is thought to be involved in the initiation of leiomyoma, and the following extracellular matrix deposition-cell proliferation, and angiogenesis-are the key cellular events implicated in leiomyoma growth (37).

Based on TCM, ULs belong to the concept of "Zhengjia" and "Jiju", which mean that they are caused by "blood stasis" and "Qi stagnation" (38).

Li et al. reviewed the effect of KBG on ULs. Among a total of 21 studies (22 experiments) involving 461 female animals, including guinea pig ($n = 20$), rats [$n = 385$, Sprague-Dawley (SD) and Wister] and mice ($n = 56$), the available evidence suggests that KBG has potentially beneficial effects over placebo on both fibroid characteristics and sex hormones in SD rats (except progesterone), Wister rats (except progesterone receptor gene expression) and Institute of Cancer Research (ICR) mice (except PR gene expression). KBG appears to reduce uterine weight and smooth muscle thickness in the guinea pig, but no data on sex hormone index was available. They concluded that that KBG may be a promising intervention for the management of uterine fibroids in animal models (39).

The effect of KBG may be related to the processes of initiation and growth, related to inflammation, fibrosis, proliferation, and angiogenesis, of ULs. Further research is expected in the future.

The Effect on Female Hormonal Dynamics

There are various results about the effect of KBG on female hormone dynamics, however, these results were very controversial.

Usuki reported several articles about the effect of KBG on hormone dynamics. One of them showed that KBG decreased estradiol-17 beta (E2) levels in media and luteinizing hormone (LH) effects on progesterone secretions, while they increased progesterone in media and LH effects on E2 secretions in rats preovulatory follicles. He concluded that KBG stimulated preovulatory follicles to secrete progesterone but to suppress E2 secretions, and it was indicated that their combination treatment with LH multiplies the sole effect of LH on E2 secretions but suppresses LH effects on progesterone (40).

Another article reported that the concentrations of E2 were significantly decreased with KBG by rat growing follicles and preovulatory follicles before a LH surge. In contrast, the levels of progesterone significantly increased with KBG by preovulatory follicles before a LH surge. These results suggested that KBG stimulated preovulatory follicles before a LH surge to secrete progesterone, but that KBG suppresses E2 secretion by growing preovulatory follicles before a LH surge (41). However, he showed different results in ovarian tissue from pregnant mare serum gonadotropin (PMS)-treated immature rats as mentioned before. He reported that administration of KBG increased the concentrations of E2, progesterone and testosterone. He concluded that KBG stimulated *in vivo* the production of E2, progesterone and testosterone by preovulatory follicles (42).

Sakamoto, et al. reported that long-term daily oral administration of KBG (300 mg/kg) in rats for 14 days decreased plasma levels of LH, follicle stimulating hormone (FSH) and E2 by 94%, 67% and 64% that of controls, respectively and KBG enhanced luteinizing hormone-releasing hormone (LH-RH)-induced increase in plasma LH and FSH levels 1.2- and 2.5-fold respectively, as compared with controls. He concluded that the results indicate that KBG may act as a LH-RH antagonist and /or a weak anti-estrogen (43). Wang et al. evaluated the estrogenic activity of these five herbal medicines and their metabolites using an estrogen receptor-dependent bioassay and an estrogen receptor-dependent reporter assay, and suggested that KBG did not exert estrogenic activity (44).

The Effect on Hot Flash by Normalizing the Attenuated CGRP Release Process

Hot flash, which is one of the symptoms of menopausal syndrome, might be described as a "the blood stasis of head and upper body." The physiological changes associated with the hot flash are different from any other flushing condition, i.e., an increased peripheral blood flow, increased heart rate, and in particular a decrease in galvanic skin resistance, which is unique to the flash (45). The evidence of various recent studies supports the role of calcitonin gene-related peptide (CGRP) as a predominant neurohormone involved in vasomotor symptoms and possibly are due to a release of this vasodilatory peptide, CGRP, from perivascular nerves (46). CGRP could act centrally on the thermoregulatory center of the hypothalamus as well as peripherally to cause vasodilation and sweating (47).

Chen et al. evaluated the relationship between CGRP and the effect of KBG on menopausal hot flash. The result was that not vasoactive intestinal peptide (VIP) but plasma CGRP, significantly elevated at the occurrence of hot flash ($P = 0.002$). Stress by cold load also significantly enhanced the over-secretion of CGRP in subjects with hot flash compared with those without hot flash ($P = 0.003$) 3 min after the load. KBG decreased plasma CGRP level in subjects with hot flash. They concluded that not vasoactive intestinal peptide (VIP) but CGRP was mainly related to the occurrence of hot flash and that KBG improves hot flash possibly by affecting plasma CGRP level (48).

Ovariectomy also triggers menopausal symptom. Ovariectomy not only potentiated CGRP-induced elevation of skin temperature and arterial vasorelaxation but also induced a lower concentration of endogenous CGRP in plasma and up-regulation of arterial CGRP receptors. It suggests that lowered CGRP in plasma due to ovarian hormone deficiency increases the number of CGRP receptors and consequently amplifies the stimulatory effects of CGRP to elevate skin temperature.

Noguchi et al. investigated the effects of 17 beta-estradiol (E2) and KBG on the release and synthesis of CGRP in ovariectomized (OVX) rats (49). Oral KBG (100–1,000 mg/kg, once a day for

7 days) restored a series of CGRP-related responses observed in OVX rats by normalizing plasma CGRP levels in a dose-dependent manner as effectively as subcutaneous injection of E2 (0.010 mg/kg, once a day for 7 days). However, KBG did not affect the lower concentration of plasma estradiol and the decreased uterine weight due to ovariectomy, although the hormone replacement of 17 beta-estradiol restored them. They concluded that these results suggested that KBG, which does not confer estrogen activity on plasma, may be useful for the treatment of hot flashes in patients for whom estrogen replacement therapy in contraindicated, as well as menopausal women (49).

Noguchi et al. also investigated the effects of E2 and KBG on the release and synthesis of CGRP in OVX rats. Ovariectomy attenuated the capsaicin-evoked increase in plasma concentration of CGRP, which was restored by treatment with subcutaneous E2 injection or KBG for 7 days after ovariectomy. However, no significant differences were observed in the CGRP concentration and the mRNA expression of dorsal root ganglia-which synthesized endogenous CGRP-in OVX rats by treating with E2 and KBG. These results suggest not only that estrogen deficiency attenuates CGRP release, but also that E2 or KBG normalizes the attenuated CGRP release process (50).

Network Pharmacology Approaches

In 2007, Hopkins created a novel concept of network pharmacology, which is built on the fundamental concept that many effective drugs in therapeutic areas act on multiple rather than single targets (51, 52). With this concept, network pharmacology can be reconstructed with molecular networks that integrate multidisciplinary concepts including biochemical, bioinformatics, and systems biology (53). In China especially, network pharmacology is increasingly applied in TCM formula research in recent years, which is identified as suitable for the study of TCM formula. Network pharmacology has become a helpful tool to achieve the interaction between the bioactive compounds and targets and the interaction between various targets, and then find out and validate the key nodes *via* network analysis and network verification. This is especially useful for multiple drug components of Chinese Herbal Medicine (54, 55).

Wang et al. reported that the results of network pharmacology research supported the premise that the potential mechanism of KBG in the treatment endometriosis might be inflammatory pathways, such as, TNF signaling pathway ($P < 0.01$) and NF-kappa B signaling pathway ($P < 0.01$) (56).

Network pharmacologic approach also indicated that the "anti-sclerotic" effect of KBG involved vascular endothelial growth factor (VEGF) signaling pathway, which relates to the process of scleroderma microvasculature, and the Toll-like receptor signaling pathway, which is a pro-fibrotic process of scleroderma (29). Since herbal medicines are composed of multiple crude drugs and have various mechanisms of action, a research method for grasping the whole picture by network pharmacology may be useful, but it is still under research.

CLINICAL RESEARCH

The following clinical research would be useful to understand the efficacy of KBG and "blood stasis." Although KBG is frequently used in cardiovascular surgery and gynecology (57), it is also used in the other departments to treat diseases related to "blood stasis." Clinical research of KBG would show that "blood stasis" might be related to the function of vascular endothelium and red blood cell deformability, and the drug could improve varicose vein, hematoma, deep vein thrombosis, rheumatoid arthritis, atopic dermatitis with lichenification, sensory symptom, hot flash, and other symptoms in pre- and postmenopausal women.

Function of Vascular Endothelium and Red Blood Cell Deformability

Nagata et al. reported that KBG improved vascular endothelial function assessed by reactive hyperemia peripheral arterial tonometry, reduced malondialdehyde which is a marker for oxidative stress and decreased serum non-esterified fatty acid in patient with metabolic syndrome related factors by controlled clinical trial (58). "Blood stasis" would be related to endotherial function, oxidative stress and arteriosclerosis induced by non-esterified fatty acid. On the other hand, Hikiami et al. reported that in patients with multiple lacunar infarctions, KBG had significant effects on red blood cell deformability as evaluated by filtration method (23), which might suggest that "blood stasis" would be related to deformation of blood cell.

Varicose Vein, Hematoma and Deep Vein Thrombosis

There are also the following reports related to the imaging of improved blood flow. Hayashi et al. reported that in patient of varicose veins of the lower extremity, KBG could improve subjective symptoms and severity of varicose veins, decrease score of "blood stasis," and increase skin perfusion pressure. The effect especially was remarkable in female (59). The concept of blood stasis also involved hematoma and deep vein thrombosis. In university hospital, KBG was used for treating hematoma in emergency department (60). Kumanomido et al. reported that KBG diminished subcutaneous hematoma after surgery in a case report (61). RCT also suggests that in elderly subjects, KBG improved deep vein thrombosis of lower limb (62).

Rheumatoid Arthritis

Nozaki et al. reported that in patients with rheumatoid arthritis, KBG showed decreased disease activity against modified disease activity score, reduced soluble vascular adhesion molecule-1 which has been postulated to be useful risk predictors of the progression of atherosclerosis and cardiovascular events, and decreased lipid peroxide. However, it did not alter CRP, IL-1β, IL-6 and TNF-α (63). This report showed that KBG might decline disease activity of rheumatoid arthritis through an antioxidative action and the prevention of atherosclerosis apart from anti-inflammatory function.

Atopic Dermatitis

The findings of fixed hard skin such as skin lesion of lichenification are also one of the criteria for blood stasis in traditional medicine. Mizawa et al. reported that KBG improved lichenification in patients of atopic dermatitis and was more effective in those having high lichenification score. On the other hand, KBG decreased LDH but did not change serum IgE and blood eosinophils, which would suggest that this drug may improve lichenification through preventing tissue injury without altering factor related to allergies (64).

Sensory Symptom

Fujita et al., reported that in patient who complained of cold sensation and numbness after cerebral stroke, KBG improved both cold sensation and numbness with visual analog scale, and increased skin temperature of diseased limb whereas did not change that of healthy limb (65). KBG might normalize sensory system or blood flow.

Hot Flash

KBG is one of the most used treatment in department of obstetrics and gynecology division. In particular, a number of reports showed KGB would be effective for hot flash. It is reported that KBG improved symptom of hot flash in young female assessed by visual analog scale (66). Moreover, Ushiroyama et al. reported that the administration of KGB decreased the blood flow under the jaw which would show signs of flash in the upper body and increased the blood flow in the lower extremities in postmenopausal women with hot flash (67). KBG was reported to be effective for hot flashes not only in female but also in male. Shigehara et al. reported that in prostate cancer patients receiving androgen deprivation therapy, KBG significantly reduced hot flash intensity, frequency and duration without the changes of prostate specific antigen and total testosterone level (68). It could be said that KBG would be effective for hot flashes related to sex hormone changes regardless of gender. On the other hand, another study reported that KBG could not change the degree of hot flash in postmenopausal women in randomized double-blind, placebo-controlled trial (69). In connection with hormonal metabolism to hot flash, Saruwatari et al. showed that KBG reduced CYP1A2, which is predominantly involved in estrogen metabolism (70). Yasui et al. reported that KBG reduced the circulating IL-8 and monocyte chemotactic protein-1 level in women with hot flashes (71). These changes of circulating hormone and chemokine might be related to improvement of the hot flash.

Endometriosis

In department of gynecology, KGB was reported to be effective to treat endometriosis (EMs). There were some reports in which KGB in combination with western medicine had achieved satisfactory curative effects. Sun XL et al. reported that KGB combined with danazol and gestrinone capsules for treating patient of EMs could reduce the immune inflammatory response in ectopic lesions and inhibit angiogenesis, and the mechanism is related to the down-regulation of VEGF and Hypoxia Inducible Factor (HIF)-1α expression (72). KBG could inhibit cell proliferation and differentiation of endometriosis, by inhibiting the MAPK/ERK kinase (MEK) and extracellular signal-regulated kinase (ERK) protein activity, blocking the cell signaling pathways, which suggesting that KBG prevented tumor growth and differentiation (73). In randomized controlled trial, KGB assisted western medicine treatment had improved the clinical symptoms and signs, and quality of life of patients of EMs, of which mechanism can be related to its inhibition of serum Leptin, VEGF, IL-8 levels and improvement of ovarian function (74). By then, KBG and mifepristone had better clinical effects on the EMs, which could reduce the serum levels of cancer antigen (CA)125 and CA199 (75). Moreover, Qian J et al. reported that the treatment using danazol with KGB had more long-lasting effect and lower recurrence rate in patients of EMs than that of danazol alone (76). KGB in combination use with western medicine was considered to be useful treatment candidate of EMs.

Others

KBG could change some symptoms of postmenopausal syndrome such as improved subjective sleep disturbances, alleviated perspiration, and reduced systolic/diastolic pressure, in pre- and postmenopausal women (77). In the premenopausal patients with uterine myomas, KBG improved clinical symptoms of hypermenorrhea and dysmenorrhea with shrinking of uterine myomas (78). All these symptoms may be related to the traditional concept of "blood stasis." Especially in Kampo-related diagnosis of "blood stasis" in clinical situation, an abdominal examination called Fukushin is fundamental skill. Yakubo et al. recently developed a clinical simulator of the examination and Arita et al. put it into practice in medical education (79, 80). Anatomical analysis of the findings of "blood stasis," such as lower abdominal resistance and fullness on abdominal examination, and its relationship with KBG could be an issue for the future.

These clinical research would suggest that KBG was applicable to various pathological conditions and changed many kinds of circulating factors seemed to be related to the mechanism, which could bridge the traditional concept of "blood stasis" and modern science.

LIMITATION

In this review, we focused on summarizing the results of basic and clinical studies so far and gaining an overview of the action of KBG. One of the limitations is that the concrete presentation of future research could not be fully shown in figures and roadmaps.

CONCLUSION

In basic research, the effects of Keishibukuryogan (KBG)/gui-zhi-fu-ling-wan (GFW), one of the most common anti-"blood stasis" prescriptions, indicated that the initiation of quantitative changes closely were related to loss of redox balances on endothelial function induced by oxidative stress, and that the following qualitative changes were related to coagulopathy, hyper viscosity; anti-platelet aggregation, lipid

metabolism; regulation of systemic leptin level and/or lipid metabolism, inflammatory factor; COX-1, 2, IL-6, 8 TNF-α, macrophage infiltration, hyperplasia, tissue fibrosis and sclerosis caused by TGF-β1 and fibronectin, dysfunction of regulated cell deaths such as, apoptosis, autophagy, ferroptosis and ovarian hormone imbalance.

Clinically, KBG, was often used for diseases related to Obstetrics and Gynecology, Endocrine Metabolism, Rheumatology and Dermatology.

In basic research, a re-verification through additional examinations of existing research and other research designs will be necessary. Since herbal medicines are composed of multiple crude drugs and have various mechanisms of action, a research method for grasping the whole picture by network pharmacology is expected to give us a bird's-eye view to provide a broader spectrum image and clarify the pathophysiology of KBG and "blood stasis." KBG is frequently used in clinical practice, and some have clear indications. Further clinical research on the prescription for "blood stasis" containing KBG is necessary based on the results of basic research.

AUTHOR CONTRIBUTIONS

KT and KC contributed to conception and writing of the manuscript. KN critically revised the manuscript. All authors contributed to this manuscript and approved the final manuscript.

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BRIEF RESEARCH REPORT
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The 2018 Japan Floods Increased the Frequency of *Yokukansan* Prescriptions Among Elderly: A Retrospective Cohort Study

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Objective: The impact of the 2018 Japan Floods on prescriptions of *Yokukansan* was evaluated.

Methods: This was a retrospective cohort study based on the National Database of Health Insurance Claims which covers all the prescriptions issued in Japan. Participants were patients aged 65 or older who received any medical care at medical institutions located in the three most-severely affected prefectures between 1 year before and after the disaster. We analyzed the number of new prescriptions of *Yokukansan* and other Kampo drugs among those who had not been prescribed any Kampo for 1 year before the disaster. Kaplan-Meier analysis and a Cox proportional hazards model were used to evaluate the risk of the disaster for a new prescription.

Results: Subjects comprised 1,372,417 people (including 12,787 victims, 0.93%). The hazard ratio (HR) of the disaster for *Yokukansan* prescriptions was 1.49 [95% confidence intervals (CI): 1.25–1.78], and 1.54 (95% CI: 1.29–1.84) in the crude and age-sex adjusted model, respectively. The HR of the disaster for prescription of other Kampo drugs in the crude and adjusted model was 1.33 (95% CI: 1.27–1.39), and 1.32 (95% CI: 1.27–1.38), respectively. The magnitude of increase of victims prescribed *Yokukansan* (31.4%) was statistically higher than for those prescribed other Kampo drugs (19.3%) ($p < 0.001$).

Conclusion: The disaster increased prescriptions of both *Yokukansan* and other Kampo drugs among elderly victims. The increase was more remarkable in *Yokukansan* than other Kampo drugs. Clinicians and policymakers should be aware of the increased need for *Yokukansan* in times of natural disaster.

Keywords: natural disaster, mental health care, Yokukansan, Kampo (traditional Japanese herbal medicine), elderly people, National Database of Health Insurance Claims, prescription, rural health services

INTRODUCTION

Global warming is causing rapid climate changes, and the scale of disasters is increasing worldwide. Therefore, it has become important around the world to investigate effective relief activities to cope with such disasters.

The 2018 Japan Floods, which occurred from June 28 to July 8, 2018, had a widespread impact across the country, especially in western Japan. The amount of financial damage was the second highest among water-related disasters in Japan. The number of deaths and missing persons was 271 (55.7% was elderly people over 65 years old) (1), which was the most since The July 1982 Torrential Rains in Nagasaki (2, 3). Total damaged houses were 29,473 including completely destroyed, half-destroyed, partially destroyed, and flooded above the floor level (1), and the total damage amounted to ∼1.41 trillion yen/12.66 billion dollars (1 dollar = 111.37 yen, converted at Central rate, average in the month, Tokyo Market as of July 2018) (4). The damage was concentrated in Okayama, Hiroshima, and Ehime prefectures; with 90.8% of deaths and missing persons, 84.9% of total damaged houses (1), and 66.1% of the total amount of damage (4) occurring in these areas.

In this study, among the numerous health problems caused by the torrential rains (5), we examined the use of the Kampo medicines for the mental symptoms of the elderly, who make up the majority of the disaster victims. It is well known that various mental disorders, for example, depression, anxiety, and post-traumatic stress disorder (PTSD), increase due to natural disasters (6). Anti-anxiety and hypnotic drugs used for such cases are associated with risks such as lightheadedness and falling, and benzodiazepines may cause dependency. Kampo medicines do not have such side effects and are safer to use, so they are used in combination with Western medicines or when Western medicines are being tapered or discontinued. In Japan, 148 Kampo formulas are covered by the public health insurance and are frequently prescribed for female and elderly persons by more than 80% of medical doctors. Kampo is useful when a disaster occurs and medical equipment cannot be used, because it is prescribed based on symptoms, inquiry, and findings. Indeed, Kampo therapy was provided in evacuation centers after the Great East Japan Earthquake and showed beneficial effects (7). It is expected that the need for Kampo therapy will rise in response to the remarkable increase in the mental problems caused by a natural disaster, but there has been no research to confirm this.

Out of 148 Kampo drugs, *Yokukansan* (YKS) is well known Kampo formula in Japan that is used to treat mental symptoms. It consists of seven kinds of medical herbs and its indications are for weakness, nervousness, insomnia, crying at night in children, and childhood neurosis. Indicated symptoms may also include agitation and irritability. YKS is listed in guidelines by the Japan Geriatrics Society as a Kampo that has been validated for efficacy and safety in the elderly (8, 9). Moreover, it has been reported in randomized controlled trials (RCTs) (10, 11) and meta-analysis (12) that YKS is effective in relieving agitation symptoms related to behavioral and psychological symptoms of dementia (BPSD), such as hallucinations, delusions, agitation, and violence. Therefore, in the guideline for dementia, it is written that atypical antipsychotics such as risperidone and aripiprazole are effective in treating agitation, and the use of YKS may also be considered (13). In addition, YKS is selected out of a number of Kampo drugs to be carried as a medicine by the Japan Medical Association Team (JMAT), which is managed by the Japan Medical Association to support victims in disaster-affected areas. Therefore, it is possible that mental health problems which could be treated by YKS arise from a natural disaster, and that YKS prescriptions increase in disaster-affected areas to improve the psychiatric condition of elderly persons.

In this study, we quantitate the usage of YKS to care the mental symptoms of the elderly persons who were affected by the disaster, by using the probability of new prescriptions of YKS as an indicator. We also clarify if the magnitude of increase of persons prescribed YKS is greater than that of other persons prescribed other 147 Kampo drugs.

METHODS

Research Design

Retrospective cohort study.

Data Source

This study is based on the NDB managed by the Ministry of Health, Labor and Welfare (permission no. 0710-4). The NDB is one of several government-maintained nationwide healthcare-related databases in Japan. All residents of Japan are covered by the public health insurance, and thus the database includes data on all the drugs prescribed in Japan.

Settings

Okayama, Hiroshima, and Ehime prefectures were selected as the target areas for the location of medical institutions, where the damage was concentrated as described in the introduction. The survey period was set between July 2017 and June 2019, which is a period of 1 year before the disaster and 1 year after the disaster. The subjects of this study were those who were 65 years old or older and visited and were issued health insurance claims from medical institutions located in Okayama, Hiroshima, and Ehime prefectures among the registrants in the database. Among the subjects, those who were identified as disaster-victims by the local government were also considered as disaster-victims in this study. The rest of the participants were identified as non-victims and analyzed separately.

Definition of Disaster-Victims

During the 2018 Japan Floods, it was announced by the Japanese government that medical insurance co-payments would be fully exempted for victims (14). We therefore defined a "disaster-victim" as a person who was listed as a disaster-victim in the special notes on health insurance claims issued after the disaster. However, people whose medical expenses had been already funded by the government, such as livelihood recipients and atomic bomb survivors who were provided with an atomic bomb survivor's certificate, were not eligible for exemption due to the disaster, therefore were categorized as non-victims even if they were affected.

Government-certified victims were fully exempted from the general out-of-pocket charge (10–30% of the total medical expense) for any medical services and were enrolled as such in the NDB. A disaster-victim was certified by the local government in their residential municipality. The criteria for a certified "disaster-victim" fell into one of the following categories: (1) residential house was completely or partially destroyed, burned down, flooded above the floor level, or similarly damaged, (2) family member who had financially supported the person was killed or suffered severely, or was missing.

Targeted Kampo Drugs

Regardless of whether a participant was affected by the disaster or not, YKS was the fourth most commonly prescribed Kampo drug both before and after the disaster (4.9–5.3%) (**Supplementary Figures 1A,B**), and it was the most commonly prescribed antipsychotic Kampo drug (27.3–28.6%) (**Supplementary Figures 1C,D**). Moreover, among 39 Kampo drugs approved by the Japan's Ministry of Health, Labor and Welfare to improve psychological symptoms, YKS is the only Kampo drug whose effectiveness is supported with sound scientific evidence (15, 16). For these reasons, we chose YKS as the subject of our analysis.

Variables

Micro analysis: The month when the new prescription of YKS was issued for the subjects was identified within the period of the study. Similarly, the month when the new prescription of other Kampo drugs was issued for the subjects was identified. Data on whether the subject was affected by the disaster, age, and gender were also extracted. However, those who had received prescriptions of any Kampo drugs before the disaster were excluded. For age categories and gender, we adopted the numbers on the first health insurance claims within the period of the study.

Aggregate analysis: In the subjects, we counted the number of people who received a prescription of YKS during the period of the study for 1 year before and after the disaster respectively. Likewise, the number of people who received prescriptions of any of 147 Kampo drugs other than YKS was also counted.

Statistical Analysis

Using micro analysis, we quantitatively examined the extent to which the disaster increased the incidence of new prescriptions of YKS and other Kampo drugs. Aggregate analysis was used to compare the change in the magnitude of increase in the number of people prescribed between YKS and other Kampo drugs before and after the disaster. The magnitude of increase was calculated for all subjects for 1 year before and 1 year after the disaster. The difference was examined by a proportion test.

Descriptive Statistics

We described the basic characteristics represented by discrete variables for victims and non-victims and conducted a χ-square test to examine the differences in the basic attributes used in the micro analysis, such as age and gender, between disaster victims and non-victims.

Examination of the Incidence of New Prescriptions Using Kaplan-Meier Failure Estimates

The new incidence rate of YKS prescriptions verses that of other Kampo drugs were described graphically with the Kaplan-Meier analysis. The start point of the observation was July 2018 and prescriptions were recorded on a monthly basis. The rate was compared between the two groups with the log-rank test.

Examination of the Impact of the Disaster on the Event Probability of New Prescriptions Using the Cox Proportional Hazards Model

We used the Cox proportional hazards model to quantitatively measure the impact of the disaster on the event probability of new prescriptions of YKS and the other Kampo drugs, respectively. The period of observation was for 1 year following July 2018. In addition to the crude model, in which the effect of the disaster was the only variate, multivariate estimation which adjusted for gender and age category was also conducted (adjusted model). After examination of the Cox proportional hazards model, we confirmed that proportional hazard assumption was clear.

We performed all statistical analyses using STATA/MP version 16 (Stata Corp, 2019).

Ethics

The need for informed consent was obviated by the anonymity of the NDB. This study was permitted by the institutional review board of Hiroshima University (No. E-1688).

RESULTS

Micro Analysis

The subjects were extracted by restricting the sample to those who had not received any prescriptions of the 148 Kampo drugs in 1 year prior to the disaster. The demographics of those subjects are presented in **Table 1**. The total number of participants aged 65 years old or more was 1,372,417, and 0.93% were disaster-victims (12,787 people). Participants in their seventies were relatively more affected compared to the other age categories ($p < 0.001$).

The percentage of all participants by gender was 44.28% male and 55.72% female, which was similar to the percentage of males and females of the same age categories who lived in Okayama, Hiroshima, and Ehime prefectures (42.71 and 57.29%, respectively) as of January 2018 (17). A gender difference in the degree of disaster vulnerability was not observed ($p = 0.822$).

Of the three prefectures, Okayama prefecture was the most common location of the medical institutions visited by victims, accounting for 45.95%. The test results showed that the probability of being affected by the disaster was significantly higher for participants who visited medical institutions in Okayama prefecture compared to the other two prefectures ($p < 0.001$).

The number of victims newly prescribed for YKS amounted to 125 (0.98% of all victims), while non-victims amounted to 8,913 (0.66% of all non-victims). The proportion of new prescriptions of YKS for the disaster-victims was significantly higher than for non-victims ($p < 0.001$).

The number of disaster-victims prescribed for other Kampo drugs amounted to 1,981 (15.49% of all victims), while non-victims amounted to 161,935 (11.91% of all non-victims). The proportion of new prescriptions of other Kampo drugs for victims was significantly higher than for non-victims ($p < 0.001$).

Kaplan-Meier failure curves for the patients newly prescribed YKS and other Kampo drugs are shown in **Figure 1**. As a result of a series of log-rank tests, new prescriptions of YKS (**Figure 1**, left panel) occurred at a higher rate in disaster-victims compared with non-victims ($p < 0.001$) and was concentrated in the 2 months after the disaster. We also estimated new prescriptions of other Kampo drugs using the same method (**Figure 1**, right panel). New prescriptions were observed more frequently among victims than non-victims, which was similar to the results for

TABLE 1 | Basic characteristics of study subjects.

Participants		**Victims**	**Non-victims**	**p-value**
		12,787 (0.93)	**1,359,630 (99.07)**	
Age classification, *n* (%)	65–69	3,358 (26.26)	373,167 (27.45)	$p < 0.001$
	70–74	3,168 (24.78)	311,035 (22.88)	
	75–79	2,440 (19.08)	237,544 (17.47)	
	80-	3,821 (29.88)	437,884 (32.21)	
Gender, *n* (%)	Male	5,649 (44.18)	602,001 (44.28)	$p = 0.822$
	Female	7,138 (55.82)	757,629 (55.72)	
Prefecture, *n* (%)	Okayama	5,876 (45.95)	407,601 (29.98)	$p < 0.001$
	Hiroshima	3,579 (27.99)	566,009 (41.63)	
	Ehime	2,700 (21.12)	316,596 (23.29)	
	Missing or other	632 (4.94)	69,424 (5.11)	
Incidence of new prescription of *Yokukansan*	Prescribed participants	125 (0.98)	8,913 (0.66)	$p < 0.001$
after disaster, *n* (%)	Non-prescribed participants	12,662 (99.02)	1,350,717 (99.34)	
Incidence of new prescription of other Kampo	Prescribed participants	1,981 (15.49)	161,935 (11.91)	$p < 0.001$
drugs after disaster, *n* (%)	Non-prescribed participants	10,806 (84.51)	1,197,695 (88.09)	

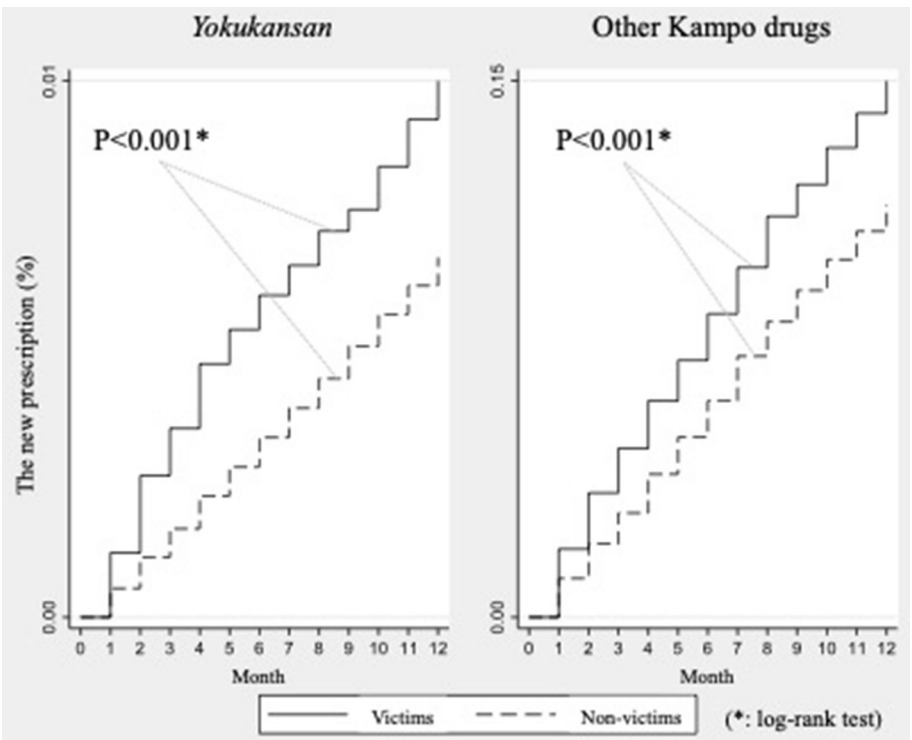

FIGURE 1 | Kaplan-Meier failure curves for the patients of newly prescribed *Yokukansan* and other Kampo drugs.

TABLE 2 | Hazard ratios and 95% confidence intervals for a new prescription **(A)** *Yokukansan,* **(B)** Other Kampo drugs.

		Hazard ratio (95% CI)	
		Crude	Adjusted
(A)			
Victims (Ref = Non-victims)		1.49 (1.25–1.78)	1.54 (1.29–1.84)
Age categories (Ref = 65–69)	70–74		1.90 (1.72–2.10)
	75–79		3.57 (3.25–3.92)
	80–		8.35 (7.69–9.08)
Gender (Ref = male)			1.07 (1.03–1.12)
(B)			
Victims (Ref = Non-victims)		1.33 (1.27–1.39)	1.32 (1.27–1.38)
Age categories (Ref = 65–69)	70–74		1.12 (1.11–1.14)
	75–79		1.26 (1.25–1.28)
	80–		1.03 (1.02–1.04)
Gender (Ref = male)			1.15 (1.14–1.16)

TABLE 3 | Magnitude of increase in the number of subjects prescribed *Yokukansan* and other Kampo Drugs from before to after the disaster.

	From July 2017 to June 2018 (A) *n*	From July 2017 to June 2018 (B) *n*	MI* (B-A)/(A) %	*p* value
Victims				
Yokukansan	226	297	31.4	<0.001**
Other Kampo drugs	3,448	4,113	19.3	
Non-victims				
Yokukansan	30,269	30,226	−0.1	<0.001**
Other Kampo drugs	416,431	412,353	−1.0	

**Magnitude of increase.*
***Proportion test compared the difference between MI of Yokukansan and other Kampo drugs.*

YKS ($p < 0.001$). In the contrast to YKS, however, a concentration of new prescriptions for other Kampo drugs at a specific time point was not observed.

Table 2A shows the results of an examination of new prescriptions for YKS using a Cox proportional hazards model. The hazard ratio (HR) for the disaster in the crude model was 1.49 (95% confidence intervals (CI): 1.25–1.78), while that of the adjusted model adjusted by age categories and gender was 1.54 (95% CI: 1.29–1.84), which indicated that the probability of new prescriptions of YKS had increased due to the disaster. We also examined the results by age categories. HR for the age group 70–74 was 1.90 (95% CI: 1.72–2.10), age group 75–79 was 3.57 (95% CI: 3.25–3.92), and age group more than 80 was 8.35 (95% CI: 7.69–9.08), which indicated that YKS was more likely to be prescribed for higher age categories. On the other hand, HR for the disaster in the crude model for other Kampo drugs was 1.33 (95% CI: 1.27–1.39), where the adjusted model was 1.32 (95% CI: 1.27–1.38), both of which were >1 as well as for YKS (**Table 2B**). Although the 95% CI for YKS and other Kampo drugs slightly overlapped, HR for YKS tended to be greater than the other Kampo drugs.

Aggregate Analysis

When the target population was expanded to include all people regardless of whether or not they had been prescribed Kampo before the disaster, the total number was 1,812,373, of which 16,396 were victims (**Table 3**). The number of patients who were prescribed YKS before and after the disaster was 226 and 297, respectively, and the magnitude of increase (31.4%) was significantly greater than that of other Kampo drugs (19.3%) ($p < 0.001$). In other words, the impact of the disaster on prescriptions was greater for YKS.

DISCUSSION

Main Findings

The 2018 Japan Floods increased prescriptions of both YKS and other Kampo drugs among elderly victims. The degree of increase in opportunities for prescribing YKS was greater than that of other Kampo drugs. Supported by the hazard model estimation adjusted for age and gender, these results were confirmed to be robust. The new prescriptions of YKS in victims were most frequently observed 2 months after the disaster. And YKS was more likely to be prescribed for higher age categories. These two characteristics were not observed in other Kampo drugs. Both YKS and other Kampo drugs were more likely to be prescribed for females, but YKS tended to be prescribed more often regardless of gender. To the best of our understanding, this is the first cohort study to quantitatively examine the impact of natural disasters on the prescriptions of Kampo.

Considerations

The locations of the medical institutions where the affected study subjects visited were, in order of the number of subjects, Okayama, Hiroshima, and Ehime prefectures. This ranking was the same as the ranking of the amount of damage: 421 billion yen/3.89 billion dollars for Okayama, 339 billion yen/3.13 billion dollars for Hiroshima, and 170 billion yen/1.57 billion dollars for Ehime prefecture.

"*Yokukansan*" means to adjust the function of "*kan*," meaning "liver" in Japanese. However, in terms of Kampo medicine, "liver" does not mean "liver" as described in Western medicine. The naming indicates a concept that includes various functions such as emotion, autonomic nervous function, function of the eyes and muscles, and regulation of blood distribution. In other words, YKS has the effect of suppressing mental tension, especially persistent anger caused by dysfunction of the "liver," hence its name (18).

In times of disaster, the Kampo that are prescribed for psychiatric symptoms as represented by YKS are safe and convenient to use when persons are dealing with an unusual and unfamiliar living environment, such as an evacuation center, because they do not have side effects such as undifferentiated dizziness as often experienced with antipsychotics. However, it is noted that although it contains a relatively small amount of glycyrrhiza (1.5 g/day), it is important to pay attention to its possible side effect of pseudohyperaldosteronism (19).

Post-disaster Mental Health Care and Yokukansan

Our study clarified that the the probability of new prescriptions of all Kampo statistically increased after the disaster. This was the case in particular for YKS, which is effective for mental symptoms such as irritation, and was more likely to be prescribed than other Kampo drugs for older patients. YKS is a commonly prescribed Kampo in general and more so it was in times of natural disaster.

Several possibilities can be raised regarding the cause of the high probability of prescriptions for YKS. First, YKS might be used for mental symptoms associated with cognitive decline such as for the observed correlation between damage to houses and cognitive decline among elderly people in the 2011 Great East Japan Earthquake (20). Next, considering the psychological and emotional aspects of the aftermath of the disaster period, well-known mental health problems include posttraumatic stress disorder (PTSD), depression, anxiety, and substance use disorder (21), and studies on anger are still limited. However, it has been reported that about 10% of the people who were highly affected by bushfire in Australia felt three times as much anger as those who were less affected (22). The results of analysis using the same sample showed that the possibilities for an increase in PTSD, depression, severe mental illness (SMI) in the aftermath of a bushfire was 19, 11, and 6%, respectively (23), which indicates that "anger" should be more considered as an important issue among these mental disorders. Our results indicate that YKS was more likely to be prescribed confirms that care for anger is necessary after a disaster.

The Mechanism of Action for Yokukansan

The neuromodulators of mental states such as anger and violence include serotonin and glutamate, along with dopamine, norepinephrine, GABA, and acetylcholine (24). There are a number of research studies on the mechanism of action of YKS involving the serotonin nervous system and the glutamate nervous system to adjust emotion. For the serotonin nervous system, pharmacological effects are exerted by acting on its receptors (25–27), and for the glutamatergic nervous system, by consequently reducing the neurotoxicity of glutamate (28–31).

Disasters and Kampo Therapy

Kampo therapy in the aftermath of large-scale disasters has been practiced in the past, mainly in Japan and China. According to a review of 12 papers on this topic, Kampo therapy has been used for primary care conditions such as common cold, constipation, insomnia, and for other conditions, for instance, irritation, PTSD, trauma, dizziness, and pain (32). It is difficult to generalize about the psychosomatic symptoms that may occur after a disaster because of differences in the season in which a disaster occurred or duration at evacuation shelters. Nevertheless, the symptoms mentioned above are considered to be medically unexplained physical symptom (MUPS), such as headache, fatigue, diarrhea, abdominal pain, and palpitations, which have been reported to persist for years after a disaster (33). Since Kampo therapy is well adapted to the treatment of MUPS, it is thought that new prescriptions increased across Kampo.

In addition, many studies show that the care needed after a disaster changes depending on the period. For example, the treatment of trauma and primary care were needed in first 2 months, while the treatment for PTSD started 6–8 months later after the disaster (32). In our study, new prescriptions of YKS were most frequent 2 months after the disaster. This observation was consistent with a previous report where the prescription sequence of YKS was eight during the first 2–4 weeks after the Great East Japan Earthquake, but second during the first 1–2 months after the earthquake among Kampo formulas prescribed (7).

Health Economics Perspective

It is also important to consider the possibility that the exemption of co-payments for medical expenses for victims might have increased the amount of drug prescriptions in general. The price elasticity of outpatient care under the medical care system for the elderly in Japan has been reported to be −0.125 to −0.076 (34). When the health insurance co-payment rate for each age group was applied to the target group of victims in this study, the average co-payment rate, calculated from the age distribution, was 18.02%. Assuming that the co-payment was totally exempted, the increase of care demand was calculated to stay at 1.37 to 2.25%. Thus, the increase in demand for medical care due to the co-payment exemption would be minimal, and the substantial increase in prescriptions of YKS and other Kampo drugs could not be explained only by price elasticity. In addition, the opportunities for prescribing YKS increased more than that for other Kampo drugs, and this difference was independent of price elasticity, suggesting that a medical condition had arisen that led to an indication for YKS in the victims.

Strengths

This study is the first to describe a change in prescriptions of YKS using completely covered data, such as health insurance claims. Japan has a universal health insurance system, which covers almost all medical practices for all residents in the country. Therefore, we were able to quantitatively measure the impact of the disaster in a manner that was close to completely covered for those that had been prescribed YKS at the doctor's discretion. In addition, the data is highly accurate because it is based on a database managed by the government.

Remaining Issues

Our study has several limitations. We were not able to examine the symptoms of individual patients and efficacy of YKS. Furthermore, medical institutions were not categorized in terms of size, department, whether or not the doctors were Kampo specialists, the content of the medical services, and accessibility. Lastly, in this study, the victims were identified based on the data from health insurance claims. However, it is presumed that there were a certain number of people who were affected, but had not applied for certification as a disaster-victim. In other words, the non-victims in this study might have included people who were

affected by the disaster, and the results obtained from this study may be lower than the actual estimates.

CONCLUSION

The number of prescriptions of both YKS and other Kampo drugs increased among elderly persons affected by a natural disaster. The increase was more remarkable for YKS than other Kampo drugs. Clinicians and policy makers should be aware of the increased need for Kampo drugs during natural disasters and need to establish a stable and smooth supply system of Kampo during normal periods to meet such needs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hiroshima University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RI, SY, and MM were involved in writing of the manuscript, contributed to conception, and design of the study. SY performed the statistical analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.777330/full#supplementary-material

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