was also reported to potentiate the responses stimulated by a β-adrenergic receptor agonist [56].

Keishibukuryogan, which is prescribed for climacteric disturbance or hot flashes, restored ovariectomy-induced effects—causing both a decrease in plasma concentration of CGRP and an increase in the number of CGRP receptors [63].

Taken together, these findings suggest that each Kampo medicine elicits its functions via certain GPCRs, contributing to the amelioration of the respective disease. Although further study is needed, we believe that Kampo medicines may cause their effects at least in part through GPCR-mediated signaling pathways. .

7. GPCR-RELATED PHARMACOLOGICAL ACTIONS AND SITES OF ACTION OF RKT

RKT, ranked third among Kampo medicines sold in Japan, is prescribed clinically for gastrointestinal disorders such as nausea, vomiting, functional dyspepsia [64], cisplatin-induced anorexia [65], as well as proton pump inhibitor-refractory and gastroesophageal reflex disease [66]. In this section, the effects of RKT are presented, with a focus on the action of RKT through GPCR, in particular the growth hormone secretagogue receptors (ghrelin receptors).

RKT is composed of 8 herbal medicines, as shown in (Table **5**): *Atractylodis Lanceae Rhizoma, Ginseng Radix, Pinelliae Tuber, Hoelen, Zizyphi Fructus, Aurantii Nobilis Pericarpium, Glycyrrhizae Radix, and Zingiberis Rhizoma*. These are mixed according to the ratio in (Table **5**) and prescribed at a dosage of 4 g/day during treatment.

Studies using animal models have shown that RKT administration causes enhanced gastric emptying and confers a protective effect against gastric mucosa injury [4]. Although the medicine is believed to be effective in treating such diseases, the mechanism by which it improves gastrointestinal functions remained to be elucidated.

Yakabi *et al.*, recently has shown that RKT ameliorates cisplatin-induced anorexia by causing an increase in circulating ghrelin concentration [67]. Ghrelin was first identified in 1999 as a 28 amino acid peptide found in rat stomach extracts [68]. Since then, extensive studies have demonstrated that, due to a variety of properties, ghrelin can stimulate growth hormone secretion, regulate energy metabolism by stimulating food intake, promote adiposity via a growth hormone-independent mechanism, and inhibit the production of anorectic proinflammatory cytokines (see review [69, 70]). The ghrelin receptors were identified before its discovery, by Smith *et al.* (1997) [71], as typical, G protein-coupled, growth hormone secretagogue receptors. The search for an endogenous ligand for this receptor was then undertaken and discovered by Kojima *et al.* (1999) [68], as mentioned above. The ghrelin receptors belong to the family of Ca^{2+}-mobilizing, $G\alpha_q$-coupled receptors [69]. Since ghrelin is the only orexigenic peptide located in the peripheral tissues, most researchers have focused on its orexigenic signaling pathways, as have Yakabi *et al.* [67]. Additionally, Fujitsuka *et al.* (2009) found that decreased contractions of the antrum and duodenum in rats treated with a selective serotonin reuptake inhibitor (SSRI) were reversed by RKT via enhancement of the circulating ghrelin concentration [72]. Because RKT activated the orexigenic ghrelin-mediated pathways, this Kampo medicine was considered to be useful in the treatment of gastrointestinal dysfunction as well as improvement of anorexia—probably by increasing ghrelin concentrations in plasma.

8. SPECIFIC GPCR TARGETS FOR RKT ACTION

1) Serotonin Receptors (5-HT_{2C} Receptor, 5-HT_{2B} Receptor)

5-HT is a multifunctional, endogenous monoamine that regulates and causes depression, anxiety-related eating disorders, vomiting, and irritable bowel syndrome [73]. It is also a key factor in adverse reactions to some widely used drugs, namely SSRIs and the anticancer agent cisplatin. Both drugs increase 5-HT levels by inhibiting its reuptake and promoting its secretion from enterochromaffin cells [4]. Recent studies have also shown that serotonergic 5-HT_2 receptors are involved in appetite control [74] and some studies have explored the mechanisms behind these effects associated with 5-HT receptors. When cisplatin or SSRIs are used in treatment, circulating 5-HT has been shown to increase; subsequently, the activation of 5-HT_{2B} receptors in gastric smooth muscles and 5-HT_{2C} receptors in the central nervous system (CNS) is promoted [56]. Some studies [75-77] have suggested that activation of both 5-HT receptors leads to decreased ghrelin levels, consequently inhibiting appetite and the motility of gastrointestinal tracts.

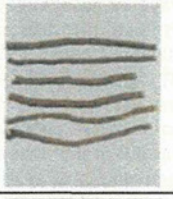
Takeda *et al.* (2008) [3] demonstrated that hesperidin, isoliquiritigenin, and heptamethoxyflavone—ingredients of *Auratii Nobilis Pericarpium* (see Table **5**) in RKT—antagonized 5-HT_{2C} and 5-HT_{2B} activities. Thus, these ingredients are thought to play important roles in the improvement of appetite caused by RKT. Administration of hesperidin reverses the decrease in plasma ghrelin in cisplatin-treated rats and shifts the fed-like motor pattern induced by SSRI administration to a fasted pattern [3]. Thus, 5-HT_{2C} antagonism by active components in RKT may lead to the improvement of anorexia. Fujitsuka *et al.* (2009) showed that oral administration of RKT restores disturbed motor activity in the gastrointestinal tract and improves anorexia in rats treated with SSRIs [72]. Intraperitoneal administration of fenfluramine or fluvoxamine shifted fasted rats from a fasted-like motor pattern in the antrum and duodenum to fed-like motor activities similar to those seen after feeding. A significant decrease in the plasma concentration of acylated ghrelin, delayed gastric emptying, and decreased food intake were also observed after SSRI administration. Concomitant oral administration of RKT with an SSRI suppressed the decrease in plasma acylated-ghrelin, changed the fed-like motor activity to fasted activity, improved anorexia, and enhanced gastric emptying. These effects were abolished by coadministration of a ghrelin receptor antagonist with RKT.

2) Growth Hormone-secretagogue Receptor, Ghrelin Receptor

As mentioned above, RKT has been shown to increase plasma ghrelin levels by inhibition of serotonergic 5-HT_{2B} and 5-HT_{2C} activities in the stomach and CNS [3, 4, 72]. Recently, Fujitsuka *et al.* (2011) demonstrated that RKT enhanced ghrelin-mediated signaling by augmenting ghrelin receptor-mediated increases in $[Ca^{2+}]_i$ [78]. In the study, ghrelin elicited an increase in $[Ca^{2+}]_i$ in ghrelin receptor-expressing COS7 cells. Although RKT had no effect on $[Ca^{2+}]_i$ in these cells, the ghrelin-induced $[Ca^{2+}]_i$ increase was enhanced by pretreatment with RKT in a concentration-dependent manner. The study's authors thought that some components of RKT might enhance the binding affinity of ghrelin to its receptor. Confirming this hypothesis, it was found that RKT enhanced the binding activity of [^{125}I]ghrelin to the ghrelin receptor [78]. The researchers then screened 43 compounds present in RKT, finding that 2 of these compounds—atractylodin and atractylodinol, which are present in *Atractylodis Lanceae Rhizoma* in RKT (Table **4**)—showed a marked increase in ghrelin/ghrelin receptor binding activity. As expected, one of these 2 compounds enhanced the ghrelin-induced increases in $[Ca^{2+}]_i$ in ghrelin receptor-expressing cells [78].

In another effect, ghrelin is reported to increase $[Ca^{2+}]_i$ in neuropeptide Y (NPY) neurons of the hypothalamic arcuate nucleus (ARC) [79], an area linked to the stimulation of feeding [80]. Fujitsuka *et al.* [78] also showed that ghrelin increased $[Ca^{2+}]_i$ in acutely isolated fura-2-loaded rat ARC neurons and that pretreatment with RKT enhanced the ghrelin-induced increase in $[Ca^{2+}]_i$. These findings indicate that RKT potentiates ghrelin's ability to increase the $[Ca^{2+}]_i$ in NPY neurons in the ARC.

Table 5. The Constituent Medicinal Herbs of Rikkunshito

Medical herbs	Total (4 g/day)		
Atractylodis Lanceae Rhizoma	0.75 g		
Ginseng Radix	0.74 g		
Pinelliae Tuber	0.74 g		
Hoelen	0.74 g		
Zizyphi Fructus	0.37 g		
Aurantii Nobilis Pericarpium	0.37 g		
Glycyrrhizae Radix	0.20 g		
Zingiberis Rhizoma	0.10 g		

Collectively, these findings suggest that the physiological functions of endogenous ghrelin are enhanced by the dual actions of RKT, namely the stimulation of ghrelin secretion (by inhibition of 5-HT_{2C} and 5-HT_{2B} receptors) and the activation of ghrelin receptor activity possibly due to allosteric changes in the receptor [78] (see Table **6**).

3) Others

Inhibition of Ghrelin Deacylating Enzyme Activity

A recent report showed that 10-gingerol, a component of *Zingiberis Rhizoma* in RKT (see Table **4**), improved cisplatin-induced anorexia by inhibiting acylated ghrelin degradation [81]. The researchers found that RKT inhibited decreases in plasma ghrelin levels by inhibiting the rate of degradation of acyl ghrelin (active form) to desacyl ghrelin (inactive form). Detailed investigations revealed that 10-gingerol inhibited the activity of ghrelin deacylating enzymes, which consequently kept active forms of acyl ghrelin at high levels in the plasma [81], as shown in (Fig. **2**).

Inhibition of Phosphodiesterase III

Intracellular cAMP levels are related to functions of appetite such as leptin-induced anorexia [82, 83]. Concentrations of intracellular cAMP levels are balanced by the opposing activities of the cAMP-synthesizing enzyme adenylyl cyclase and the degrading enzyme phosphodiesterases. The latter are subdivided into 11 broad

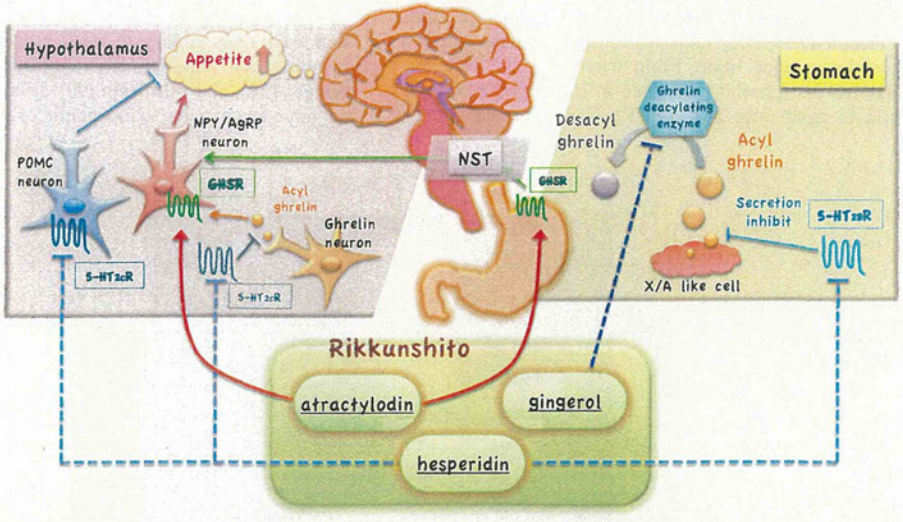


Fig. (2). Schematic diagram of mechanisms of action for the signaling pathways induced by each ingredient of rikkunshito, as related to effects on appetite. Hesperidin inhibits 5-HT_{2B} receptors both in the stomach and the hypothalamus. Atractylodin activates ghrelin receptors in the stomach and in the hypothalamus. Gingerol inhibits ghrelin deacylating enzymes in various tissues, including the stomach. POMC: proopiomelanocortin, GHSR: ghrelin receptor, NST: nucleus tractus solitarii, NPY: neuropeptide Y, AgRP: agouti-related peptide.

Table 6. Effects of Rikkunshito on GPCRs and it's Pharmacological Actions.

Target GPCRs of Rikkunshito	Pharmacological Actions	Mechanism of Actions	References
5-HT_{2B} receptor	Improvement of anorexia	Enhancement of acylated-ghrelin release by inhibition of the receptor	[3]
5-HT_{2C} receptor	Improvement of anorexia	Increasement of ghrelin secretion from the hypothalamus by inhibition of the receptor	[67]
	Improvement of gastrointestinal dysmotility	Inhibition of the receptor activity	[72, 78]
	Decrease of anxiety-related behavior		[78]
Growth hormone secretagogue receptor (Ghrelin receptor)	Improvement of anorexia	Enhancement of ghrelin receptor signaling	[78]
		Recovery of decreased hypothalamic ghrelin receptor 1a mRNA expression	[123]
	Prolonged survival in animals and patients with cancer	Activation of ghrelin receptors	[78]
	Improvement of anorexia	Inhibition of ghrelin degrading enzyme from acyl ghrelin to desacyl ghrelin	[81]
		Amelioration of ghrelin receptor activity via phosphodiesterase III inhibition	[85]

families based on their distribution in tissues, biochemical properties, and sensitivity to chemical inhibitors [84]. In particular, phosphodiesterase III is activated by the anorexigenic peptide leptin [82, 83]. Kohno *et al.* (2007) [80] found that leptin suppressed ghrelin-stimulated food intake and that this effect was abolished by administration of inhibitors of phosphodiesterase III. Takeda *et al.* (2010) [85] reported that food intake in aged mice was significantly lower than in young mice. In these older mice, researchers found that RKT ameliorated aging-associated anorexia, although ghrelin administration failed to recover normal food intake levels. This finding suggests that ghrelin caused resistance to appetite control in aged mice. The researchers also found that application of a phosphodiesterase III inhibitor increased food intake in such mice. Some components of RKT—namely, nobiletin, isoliquiritigenin, and heptamethoxyflavone, which are present in *Aurantii Nobilis Pericarpium* in RKT (Table **4**)—indeed inhibited anorexic effects and increased food intake in aged mice. From these "results", the researchers concluded that ghrelin resistance in the appetite-control system occurred in aged mice and that RKT ameliorated aging-associated anorexia via phosphodiesterase III inhibition (see Table **6**).

9. NEW ASSAY METHOD FOR DETECTING GPCR TARGETS OF KAMPO EXTRACTS

Electrical biosensors, also known as impedance-based biosensors, consist of a substrate, an electrode, and a cell layer in close contact with the electrode (Fig. **3A**). Giaever and Keese of General Electric first reported the use of impedance to measure cellular processes [86]. In their early studies, fibroblasts cultured on thin-film, gold electrodes were found to impede the flow of a very weak alternating current. The resulting change in impedance could be monitored in real-time, and the fluctuation of impedance depended on ATP concentration and actin polymerization, thus linking this change to cellular motion [87]. Since then, electrical-based detections have been applied to study a wide variety of cellular events, including cell adhesion and movement [88], cell morphological changes [89], and cell death [90]. It is now generally accepted that the impedance value corresponds to the sum of cellular events,

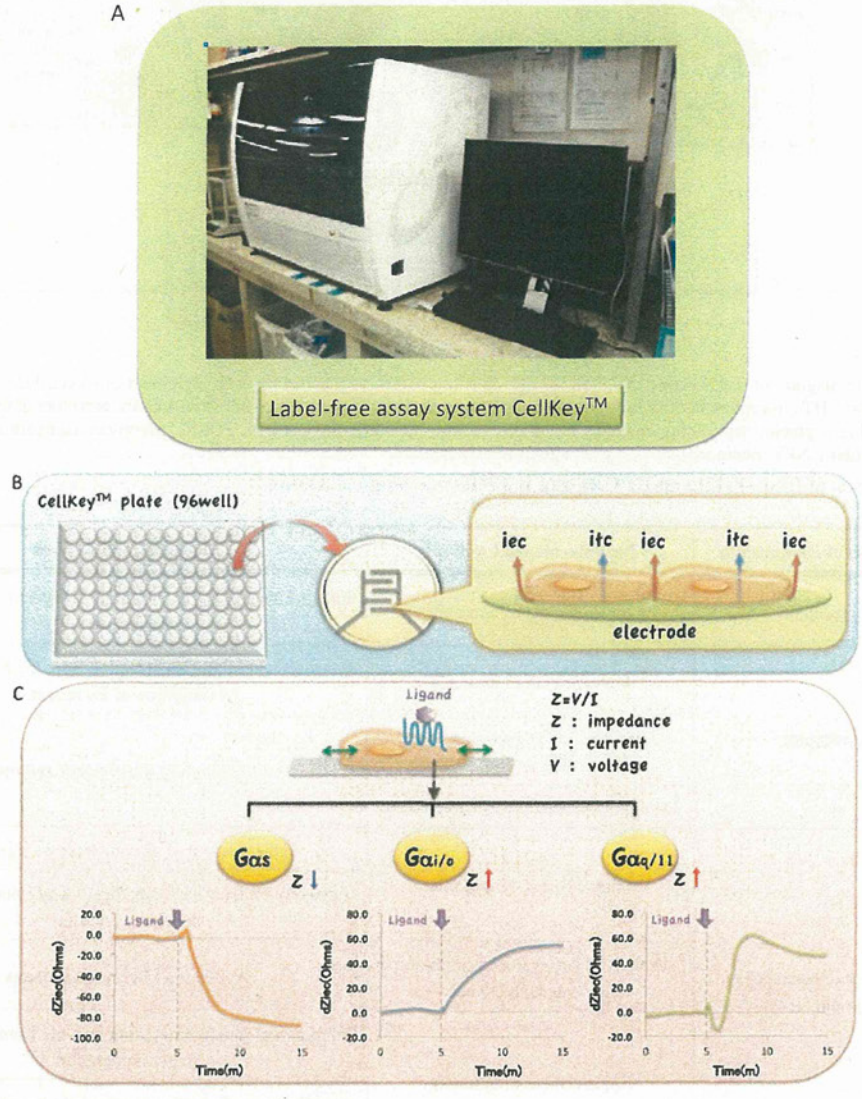


Fig. (3). A: Photograph of the label-free assay system CellKey™ equipped with operation machine and controlling computer. **B:** Principles of CellKey™ impedance assay. **C:** Schematic diagram of impedance assay with cells and representative waves mediated by typical $G\alpha_s$-, $G\alpha_{i/o}$-, and $G\alpha_{q/11}$-coupled receptors. iec: induced extracellular currents, itc: induced transcellular currents.

including the relative density of cells over the electrode surface and the relative adherence of these cells.

A high-throughput system, CellKey™, developed by MDS Analytical Technologies, was designed to detect acute cellular responses in 96- and 384-well formats (Fig. **3A**). Based on the CellKey™ system, others and we have observed distinct response profiles depending on the G-protein pathway that is activated [91-93]. Many studies with GPCR-ligand sets have demonstrated similar rank-order potency values between CellKey™ impedance and traditional cAMP or Ca^{2+} assays [94-96]. A schematic diagram of the measurement of several types GPCR-mediated signaling is shown in (Figs. **3B** and **3C**).

Our preliminary results using COS7 cells stably expressing rat ghrelin receptors are shown in (Fig. **4**), along with the CellKey™ assay system. As shown, varying concentrations of ghrelin were applied to 96-well plates seeding the COS7 cells; their cellular-impedance changes were then measured with the assay system. Impedance currents were increased in a concentration-dependent manner, with proportional changes of notch (within the purple dash line), which have a shape typical of the $G\alpha_{q/11}$-coupled, Ca^{2+}-mobilizing receptors [94-96]. The shape of concentration-response curves are almost the same if data are taken from the "Notch" portions or the next-peak portions (within the orange dash line) (Fig. **4**). Cells stably expressing $G\alpha_{i/o}$-coupled human μ-opioid receptors and cells expressing $G\alpha_s$-coupled human adrenergic β_2 receptors were also tested. The expected patterns of impedance waves were obtained when the appropriate concentrations of (10^{-8} M each) μ-opioid receptor-specific agonist [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO) and adrenergic β receptor-specific agonist isoproterenol (data not shown) were applied.

When RKT (100 μg/ml) is simultaneously added together with varying concentrations of ghrelin (10^{-11}–10^{-7}M), robust enhancement of the impedance was observed (unpublished data), although RKT by itself did not cause any impedance-based currents (unpublished data), demonstrating that RKT actually modified and enhanced the ghrelin-induced receptor activation. We are now testing a series of ingredients in RKT with the same system. Likewise, using the CellKey™ assay system, the actual ingredients that mimic Kampo medicines and affect cellular signaling can be identified in the future.

10. PERSPECTIVES

Data about the pharmacological actions and detailed signaling pathways of the Kampo medicines continue to accumulate. Recent progress in the use of fine assays for detecting and screening GPCR signals, i.e., with the recently developed CellKey™ system, continues to aid in the accumulation of more data. It is becoming apparent that identification of specific ingredients of the Kampo medicines is very important for understanding the medicines' actual mechanisms of action and in the further development of useful drugs based on these identified ingredients. Thus, it is important to identify the pure formulae of such compounds. To identify such substances from mixtures of Kampo medicines, rapid and high-throughput assay systems, such as the CellKey™ system, are needed.

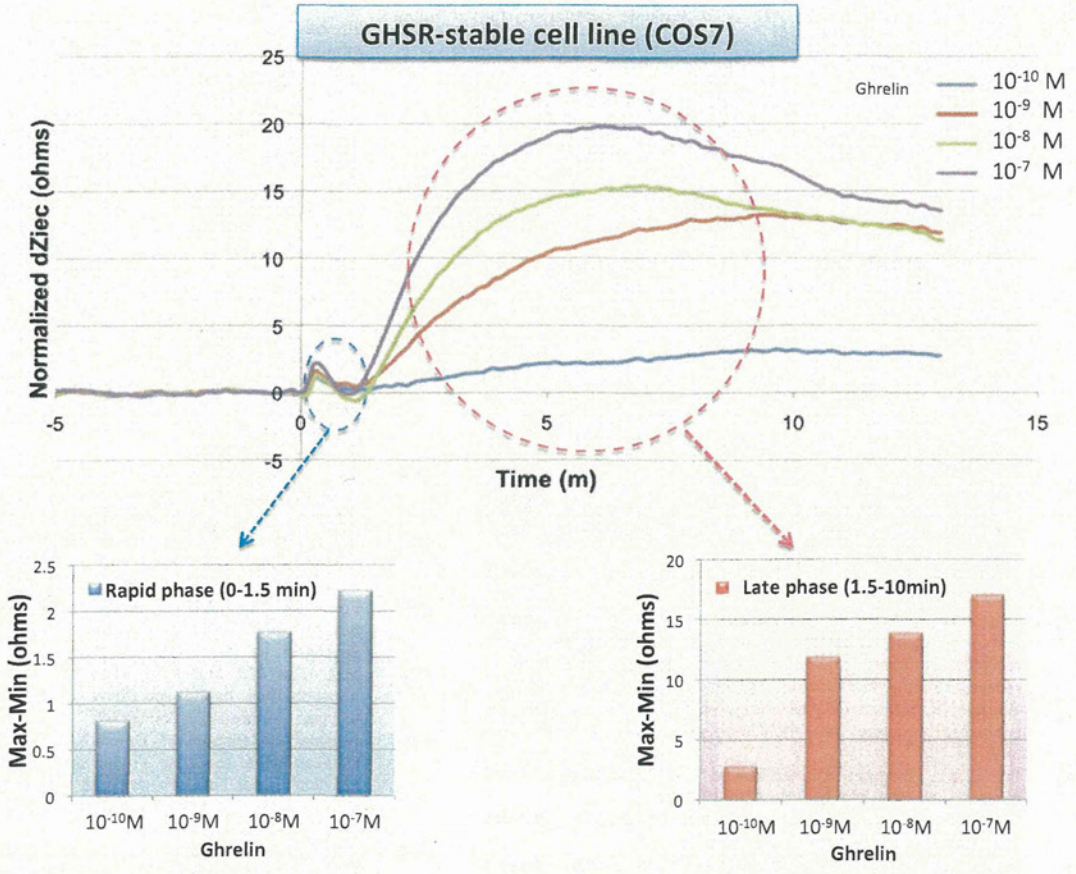


Fig. (4). Representative pattern of extracellular impedance currents induced by indicated concentrations of ghrelin in COS7 cells stably expressing rat ghrelin receptors. Concentration-response curves of ghrelin-induced waves at the "notch" points (left) and second phases of peak waves (right).

In conclusion, although herbal medicines, including Kampo, have been gradually recognized as a beneficial form of treatment, further scientific evidence and certification of Kampo's effectiveness is warranted to encourage its worldwide use.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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REFERENCES

[1] Yu F, Takahashi T, Moriya J, *et al.* Traditional Chinese medicine and Kampo: a review from the distant past for the future. J Int Med Res 2006; 34:231-9.

[2] Mizukami K, Asada T, Kinoshita T, *et al.* A randomized cross-over study of a traditional Japanese medicine (kampo), yokukansan, in the treatment of the behavioural and psychological symptoms of dementia. Int J Neuropsychopharmacol 2009; 12:191-9.

[3] Takeda H, Sadakane C, Hattori T, *et al.* Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. Gastroenterology 2008; 134:2004-13.

[4] Hattori T. Rikkunshito and ghrelin. Int J Pept 2010; PII: 283549.

[5] Agnati LF, Guidolin D, Leo G, *et al.* Possible new targets for GPCR modulation: allosteric interactions, plasma membrane domains, intercellular transfer and epigenetic mechanisms. J Recep Signal Trans Res 2011; 31:315-31.

[6] Klabunde T, Hessler G. Drug design strategies for targeting G-protein-coupled receptors. Chembiochem 2002; 3:928-44.

[7] CSR Report. TSUMURA Corporate Social Responsibility Report 2011:1-54.

[8] Satoh K, Hashimoto K, Hayakawa T, *et al.* Mechanism of atropine-resistant contraction induced by Dai-kenchu-to in guinea pig ileum. Jpn J Pharmacol 2001; 86:32-7.

[9] Arakawa T, Higuchi K, Fujiwara Y, *et al.* Gastroprotection by Liu-Jun-Zi-Tang (TJ-43): possible mediation of nitric oxide but not prostaglandins or sulfhydryls. Drugs Exp Clin Res 1999; 25:207-10.

[10] Kido T, Nakai Y, Kase Y, *et al.* Effects of rikkunshi-to, a traditional Japanese medicine, on the delay of gastric emptying induced by N(G)-nitro-L-arginine. J Pharmacol Sci 2005; 98:161-7.

[11] Kawakami Z, Ikarashi Y, Kase Y. Isoliquiritigenin is a novel NMDA receptor antagonist in kampo medicine yokukansan. Cell Mol Neurobiol 2011; 31:1203-12.

[12] Egashira N, Nogami A, Iwasaki K, *et al.* Yokukansan Enhances Pentobarbital-Induced Sleep in Socially Isolated Mice: Possible Involvement of GABA(A) - Benzodiazepine Receptor Complex. J Pharmacol Sci 2011; 116:316-20.

[13] Foord SM. Receptor classification: post genome. Curr Opin Pharmacol 2002; 2:561-6.

[14] Kristiansen K. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. Pharmacol Ther 2004; 103:21-80.

[15] Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: conserved structure and molecular mechanism. Nature 1991; 349:117-27.

[16] Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat Rev Mol Cell Biol 2002; 3:639-50.

[17] Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. Science 2005; 308:512-7.

[18] Bylund DB, Toews ML. Radioligand binding methods: practical guide and tips. Am J Physiol 1993; 265:L421-9.

[19] Milligan G. Principles: extending the utility of [35S]GTP gamma S binding assays. Trends Pharmacol Sci 2003; 24:87-90.

[20] Harrison C, Traynor JR. The [35S]GTPgammaS binding assay: approaches and applications in pharmacology. Life Sci 2003; 74:489-508.

[21] Emkey R, Rankl NB. Screening G protein-coupled receptors: measurement of intracellular calcium using the fluorometric imaging plate reader. Methods Mol Biol 2009; 565:145-58.

[22] Itoh T, Yamakawa J, Mai M, Yamaguchi N, Kanda T. The effect of the herbal medicine dai-kenchu-to on post-operative ileus. J Int Med Res 2002; 30:428-32.

[23] Ohya T, Usui Y, Arii S, Iwai T, Susumu T. Effect of dai-kenchu-to on obstructive bowel disease in children. Am J Chin Med 2003; 31:129-35.

[24] Kono T, Kanematsu T, Kitajima M. Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines: is it time yet? Surgery 2009; 146:837-40.

[25] Fukuda H, Chen C, Mantyh C, Ludwig K, Pappas TN, Takahashi T. The herbal medicine, Dai-Kenchu-to, accelerates delayed gastrointestinal transit after the operation in rats. J Surg Res 2006; 131:290-5.

[26] Tokita Y, Yuzurihara M, Sakaguchi M, Satoh K, Kase Y. The pharmacological effects of Daikenchuto, a traditional herbal medicine, on delayed gastrointestinal transit in rat postoperative ileus. J Pharmacol Sci 2007; 104:303-10.

[27] Tokita Y, Satoh K, Sakaguchi M, *et al.* The preventive effect of Daikenchuto on postoperative adhesion-induced intestinal obstruction in rats. Inflammopharmacology 2007; 15:65-6.

[28] Satoh K, Kase Y, Yuzurihara M, Mizoguchi K, Kurauchi K, Ishige A. Effect of Dai-kenchu-to (Da-Jian-Zhong-Tang) on the delayed intestinal propulsion induced by chlorpromazine in mice. J Ethnopharmacol 2003; 86:37-44.

[29] Satoh K, Hayakawa T, Kase Y, *et al.* Mechanisms for contractile effect of Dai-kenchu-to in isolated guinea pig ileum. Dig Dis Sci 2001; 46:250-6.

[30] Jin XL, Shibata C, Naito H, *et al.* Intraduodenal and intrajejunal administration of the herbal medicine, dai-kenchu-tou, stimulates small intestinal motility via cholinergic receptors in conscious dogs. Dig Dis Sci 2001; 46:1171-6.

[31] Nagano T, Itoh H, Takeyama M. Effect of Dai-kenchu-to on levels of 3 brain-gut peptides (motilin, gastrin and somatostatin) in human plasma. Biol Pharm Bull 1999; 22:1131-3.

[32] Nagano T, Itoh H, Takeyama M. Effects of Dai-kenchu-to on levels of 5-hydroxytryptamine (serotonin) and vasoactive intestinal peptides in human plasma. Biol Pharm Bull 2000; 23:352-3.

[33] Sato Y, Katagiri F, Inoue S, Itoh H, Takeyama M. Dai-kenchu-to raises levels of calcitonin gene-related peptide and substance P in human plasma. Biol Pharm Bull 2004; 27:1875-7.

[34] Sato Y, Inoue S, Katagiri F, Itoh H, Takeyama M. Effects of pirenzepine on Dai-kenchu-to-induced elevation of the plasma neuropeptide levels in humans. Biol Pharm Bull 2006; 29:166-71.

[35] Murata P, Kase Y, Ishige A, Sasaki H, Kurosawa S, Nakamura T. The herbal medicine Dai-kenchu-to and one of its active components [6]-shogaol increase intestinal blood flow in rats. Life Sci 2002; 70:2061-70.

[36] Kono T, Koseki T, Chiba S, *et al.* Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide and receptor-activity modifying protein 1. J Surg Res 2008; 150:78-84.

[37] Kono T, Kaneko A, Hira Y, *et al.* Anti-colitis and -adhesion effects of daikenchuto via endogenous adrenomedullin enhancement in Crohn's disease mouse model. J Crohns Colitis 2010; 4:161-70.

[38] Foord SM, Marshall FH. RAMPs: accessory proteins for seven transmembrane domain receptors. Trends Pharmacol Sci 1999; 20:184-7.

[39] McLatchie LM, Fraser NJ, Main MJ, *et al.* RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 1998; 393:333-9.

[40] Iwasaki K, Maruyama M, Tomita N, *et al.* Effects of the traditional Chinese herbal medicine Yi-Gan San for cholinesterase inhibitor-resistant visual hallucinations and neuropsychiatric symptoms in patients with dementia with Lewy bodies. J Clin Psychiatry 2005; 66:1612-3.

[41] Monji A, Takita M, Samejima T, *et al.* Effect of yokukansan on the behavioral and psychological symptoms of dementia in elderly patients with Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33:308-11.

[42] Ikarashi Y, Iizuka S, Imamura S, *et al.* Effects of yokukansan, a traditional Japanese medicine, on memory disturbance and

behavioral and psychological symptoms of dementia in thiamine-deficient rats. Biol Pharm Bull 2009; 32:1701-9.

[43] Iizuka S, Kawakami Z, Imamura S, *et al.* Electron-microscopic examination of effects of yokukansan, a traditional Japanese medicine, on degeneration of cerebral cells in thiamine-deficient rats. Neuropathology 2010; 30: 524-36.

[44] Tamano H, Kan F, Oku N, Takeda A. Ameliorative effect of Yokukansan on social isolation-induced aggressive behavior of zinc-deficient young mice. Brain Res Bull 2010; 83:351-5.

[45] Sekiguchi K, Yamaguchi T, Tabuchi M, Ikarashi Y, Kase Y. Effects of yokukansan, a traditional Japanese medicine, on aggressiveness induced by intracerebroventricular injection of amyloid beta protein into mice. Phytother Res 2009; 23:1175-81.

[46] Sekiguchi K, Imamura S, Yamaguchi T, *et al.* Effects of yokukansan and donepezil on learning disturbance and aggressiveness induced by intracerebroventricular injection of amyloid Œ≤ protein in mice. Phytother Res 2011; 25:501-7.

[47] Tabuchi M, Yamaguchi T, Iizuka S, Imamura S, Ikarashi Y, Kase Y. Ameliorative effects of yokukansan, a traditional Japanese medicine, on learning and non-cognitive disturbances in the Tg2576 mouse model of Alzheimer's disease. J Ethnopharmacol 2009; 122:157-62.

[48] Kanno H, Sekiguchi K, Yamaguchi T, *et al.* Effect of yokukansan, a traditional Japanese medicine, on social and aggressive behaviour of para-chloroamphetamine-injected rats. J Pharm Pharmacol 2009; 61:1249-56.

[49] Terawaki K, Ikarashi Y, Sekiguchi K, Nakai Y, Kase Y. Partial agonistic effect of yokukansan on human recombinant serotonin 1A receptors expressed in the membranes of Chinese hamster ovary cells. J Ethnopharmacol 2010; 127:306-12.

[50] Ueda T, Ugawa S, Ishida Y, Shimada S. Geissoschizine methyl ether has third-generation antipsychotic-like actions at the dopamine and serotonin receptors. Eur J Pharmacol 2011; 671:79-86.

[51] Egashira N, Iwasaki K, Ishibashi A, *et al.* Repeated administration of Yokukansan inhibits DOI-induced head-twitch response and decreases expression of 5-hydroxytryptamine (5-HT)2A receptors in the prefrontal cortex. Prog Neuropsychopharmacol Biol Psychiatry 2008; 32:1516-20.

[52] Yamada M, Hayashida M, Zhao Q, *et al.* Ameliorative effects of yokukansan on learning and memory deficits in olfactory bulbectomized mice. J Ethnopharmacol 2011; 135:737-46.

[53] Suzuki Y, Goto K, Ishige A, Komatsu Y, Kamei J. Antinociceptive effect of Gosha-jinki-gan, a Kampo medicine, in streptozotocin-induced diabetic mice. Jpn J Pharmacol 1999; 79:169-75.

[54] Gotoh A, Goto K, Sengoku A, *et al.* Inhibition mechanism of Gosha-jinki-gan on the micturition reflex in rats. J Pharmacol Sci 2004; 96:115-23.

[55] Isohama Y, Kurita K, Kai H, Takahama K, Miyata T. Bakumondo-to (Mai-Men-Dong-Tang) increases Œ≤1-adrenergic receptor mRNA expression in rat alveolar type 2 cells. J Tradit Med 2001; 18:8-14.

[56] Tamaoki J, Chiyotani A, Takeyama K, Kanemura T, Sakai N, Konno K. Potentiation of beta-adrenergic function by saiboku-to and bakumondo-to in canine bronchial smooth muscle. Jpn J Pharmacol 1993; 62:155-9.

[57] Omiya Y, Suzuki Y, Yuzurihara M, *et al.* Antinociceptive effect of shakuyakukanzoto, a Kampo medicine, in diabetic mice. J Pharmacol Sci 2005; 99:373-80.

[58] Das AK, Mizuguchi H, Kodama M, *et al.* Sho-seiryu-to suppresses histamine signaling at the transcriptional level in TDI-sensitized nasal allergy model rats. Allergol Int 2009; 58:81-8.

[59] Sakaguchi M, Mase A, Iizuka A, *et al.* Further pharmacological study on Sho-seiryu-to as an antiallergic. Methods Find Exp Clin Pharmacol 1997; 19:707-13.

[60] Ikarashi Y, Yuzurihara M, Sakakibara I, Takahashi A, Ishimaru H, Maruyama Y. Effects of an oriental herbal medicine, "Saiboku-to", and its constituent herbs on Compound 48/80-induced histamine release from peritoneal mast cells in rats. Phytomedicine 2001; 8:8-15.

[61] Kobayashi I, Hamasaki Y, Sato R, *et al.* Saiboku-To, a herbal extract mixture, selectively inhibits 5-lipoxygenase activity in leukotriene synthesis in rat basophilic leukemia-1 cells. J Ethnopharmacol 1995; 48:33-41.

[62] Nakamura T, Kuriyama M, Kosuge E, Ishihara K, Ito K. Effects of saiboku-to (TJ-96) on the production of platelet-activating factor in human neutrophils. Ann N Y Acad Sci 1993; 685:572-9.

[63] Noguchi M, Ikarashi Y, Yuzurihara M, *et al.* Effects of the Japanese herbal medicine Keishi-bukuryo-gan and 17beta-estradiol on calcitonin gene-related peptide-induced elevation of skin temperature in ovariectomized rats. J Endocrinol 2003; 176:359-66.

[64] Arai M, Matsumura T, Tsuchiya N, *et al.* Rikkunshito improves the symptoms in patients with functional dyspepsia, accompanied by an increase in the level of plasma ghrelin. Hepatogastroenterology 2012; 59:62-6.

[65] Ohno T, Yanai M, Ando H, *et al.* Rikkunshito, a traditional Japanese medicine, suppresses cisplatin-induced anorexia in humans. Clin Exp Gastroenterol 2011; 4:291-6.

[66] Tominaga K, Iwakiri R, Fujimoto K, *et al.* Rikkunshito improves symptoms in PPI-refractory GERD patients: a prospective, randomized, multicenter trial in Japan. J Gastroenterol 2011; 47:284-92

[67] Yakabi K, Sadakane C, Noguchi M, *et al.* Reduced ghrelin secretion in the hypothalamus of rats due to cisplatin-induced anorexia. Endocrinology 2010; 151:3773-82.

[68] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402:656-60.

[69] Kojima M, Kangawa K. Ghrelin: structure and function. Physiol Rev 2005; 85:495-522.

[70] Chen CY, Asakawa A, Fujimiya M, Lee SD, Inui A. Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev 2009; 61:430-81.

[71] Smith RG, Van der Ploeg LH, Howard AD, *et al.* Peptidomimetic regulation of growth hormone secretion. Endocr Rev 1997; 18:621-45.

[72] Fujitsuka N, Asakawa A, Hayashi M, *et al.* Selective serotonin reuptake inhibitors modify physiological gastrointestinal motor activities via 5-HT2c receptor and acyl ghrelin. Biol Psychiatry 2009; 65:748-59.

[73] Pytliak M, Vargova V, Mechirova V, Felsoci M. Serotonin receptors - from molecular biology to clinical applications. Physiol Res 2011; 60:15-25.

[74] De Vry J, Schreiber R. Effects of selected serotonin 5-HT(1) and 5-HT(2) receptor agonists on feeding behavior: possible mechanisms of action. Neurosci Biobehav Rev 2000; 24:341-53.

[75] Nonogaki K, Nozue K, Oka Y. Hyperphagia alters expression of hypothalamic 5-HT2C and 5-HT1B receptor genes and plasma des-acyl ghrelin levels in Ay mice. Endocrinology 2006; 147:5893-900.

[76] Nonogaki K. Ghrelin and feedback systems. Vitam Horm 2008; 77:149-70.

[77] Taniguchi H, Ariga H, Zheng J, *et al.* Endogenous ghrelin and 5-HT regulate interdigestive gastrointestinal contractions in conscious rats. Am J Physiol Gastrointest Liver Physiol 2008; 295:G403-11.

[78] Fujitsuka N, Asakawa A, Uezono Y, *et al.* Potentiation of ghrelin signaling attenuates cancer anorexia,Äicachexia and prolongs survival. Trans Psychiatry 2011; 1:1-10.

[79] Kohno D, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca2+ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. Diabetes 2003; 52:948-56.

[80] Kohno D, Nakata M, Maekawa F, *et al.* Leptin suppresses ghrelin-induced activation of neuropeptide Y neurons in the arcuate nucleus via phosphatidylinositol 3-kinase- and phosphodiesterase 3-mediated pathway. Endocrinology 2007; 148:2251-63.

[81] Sadakane C, Muto S, Nakagawa K, *et al.* 10-Gingerol, a component of rikkunshito, improves cisplatin-induced anorexia by inhibiting acylated ghrelin degradation. Biochem Biophys Res Commun 2011; 412:506-11.

[82] Zhao AZ, Bornfeldt KE, Beavo JA. Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. J Clin Invest 1998; 102:869-73.

[83] Zhao AZ, Shinohara MM, Huang D, *et al.* Leptin induces insulin-like signaling that antagonizes cAMP elevation by glucagon in hepatocytes. J Biol Chem 2000; 275:11348-54.

[84] Yan C, Miller CL, Abe J. Regulation of phosphodiesterase 3 and inducible cAMP early repressor in the heart. Circ Res 2007; 100:489-501.

[85] Takeda H, Muto S, Hattori T, *et al.* Rikkunshito ameliorates the aging-associated decrease in ghrelin receptor reactivity via phosphodiesterase III inhibition. Endocrinology 2010; 151:244-52.
[86] Giaever I, Keese CR. Monitoring fibroblast behavior in tissue culture with an applied electric field. Proc Natl Acad Sci U S A 1984; 81:3761-4.
[87] Giaever I, Keese CR. Micromotion of mammalian cells measured electrically. Proc Natl Acad Sci USA 1991; 88:7896-900.
[88] Tiruppathi C, Malik AB, Del Vecchio PJ, Keese CR, Giaever I. Electrical method for detection of endothelial cell shape change in real time: assessment of endothelial barrier function. Proc Natl Acad Sci USA 1992; 89:7919-23.
[89] Giaever I, Keese CR. A morphological biosensor for mammalian cells. Nature 1993; 366:591-2.
[90] Zhu J, Wang X, Xu X, Abassi YA. Dynamic and label-free monitoring of natural killer cell cytotoxic activity using electronic cell sensor arrays. J Immunol Methods 2006; 309:25-33.
[91] Fang Y, Li G, Ferrie AM. Non-invasive optical biosensor for assaying endogenous G protein-coupled receptors in adherent cells. J Pharmacol Toxicol Methods 2007; 55:314-22.
[92] Peters MF, Vaillancourt F, Heroux M, Valiquette M, Scott CW. Comparing label-free biosensors for pharmacological screening with cell-based functional assays. Assay Drug Dev Technol 2010; 8:219-27.
[93] Leung G, Tang H, McGuinness R, Verdonk E, Michelotti M, Liu V. Cellular dielectric spectroscopy: A label-free technology for drug discovery. J Lab Autom 2005; 10:258-69.
[94] Ciambrone GJ, Liu VF, Lin DC, McGuinness RP, Leung GK, Pitchford S. Cellular dielectric spectroscopy: a powerful new approach to label-free cellular analysis. J Biomol Screen 2004; 9:467-80.
[95] Peters MF, Knappenberger KS, Wilkins D, *et al.* Evaluation of cellular dielectric spectroscopy, a whole-cell, label-free technology for drug discovery on Gi-coupled GPCRs. J Biomol Screen 2007; 12:312-9.
[96] McGuinness RP, Proctor JM, Gallant DL, *et al.* Enhanced selectivity screening of GPCR ligands using a label-free cell based assay technology. Comb Chem High Throughput Screen 2009; 12:812-23.
[97] Mori K, Kido T, Daikuhara H, *et al.* Effect of Hochu-ekki-to (TJ-41), a Japanese herbal medicine, on the survival of mice infected with influenza virus. Antiviral Res 1999; 44:103-11.
[98] Li T, Tamada K, Abe K, *et al.* The restoration of the antitumor T cell response from stress-induced suppression using a traditional Chinese herbal medicine Hochu-ekki-to (TJ-41:Bu-Zhong-Yi-Qi-Tang). Immunopharmacology 1999; 43:11-21.
[99] Kurose I, Miura S, Fukumura D, Suzuki H, Tsuchiya M, Ishii H. Inhibitions of platelet-activating factor production and granulocyte oxidative activation by Rikkunshi-To in the process of gastric mucosal injury¬†. Pathophysiology 1995; 2:153-9.
[100] Tamaki K, Otaka M, Shibuya T, *et al.* Traditional harbel medicine, Rikkunshito, induced HSP60 and enhances cytoprotection of small intestinal mucosal cell as a nontoxic chaperone inducer. Evid-Based Comp Alter Med 2012:1-7.
[101] Ono K, Saito K, Saeki T, Iwasaki A, Arakawa Y. Gastritis.Experimental study on inhibitory effect of acid secretion with Chinese medicine. Prog Med 1993; 13:2832-5.
[102] Takeda A, Tamano H, Itoh H, Oku N. Attenuation of abnormal glutamate release in zinc deficiency by zinc and Yokukansan. Neurochem Int 2008; 53:230-5.
[103] Kawakami Z, Kanno H, Ueki T, *et al.* Neuroprotective effects of yokukansan, a traditional Japanese medicine, on glutamate-mediated excitotoxicity in cultured cells. Neuroscience 2009; 159:1397-407.
[104] Mizowaki M, Toriizuka K, Hanawa T. Anxiolytic effect of Kami-Shoyo-San (TJ-24) in mice: possible mediation of neurosteroid synthesis. Life Sci 2001; 69:2167-77.
[105] Suzuki Y, Goto K, Ishige A, Komatsu Y, Kamei J. Effects of gosha-jinki-gan, a kampo medicine, on peripheral tissue blood flow in streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 1998; 20:321-8.
[106] Hattori T, Maruyama H, Nishimura H, *et al.* Effects of Saireito, a Japanese herbal medicine, on edema via antagonistic actions against aldosterone in anti-GBM nephritic rats. Clin Exp Nephrol 2006; 10:13-8.
[107] Kaneko T, Chiba H, Horie N, *et al.* Inhibition of prostaglandin E2 production by flavone and its related compounds. In Vivo 2010; 24:55-8.
[108] Kaneko T, Chiba H, Horie N, *et al.* Effect of Scutellariae radix ingredients on prostaglandin E(2) production and COX-2 expression by LPS-activated macrophage. In Vivo 2009; 23:577-81.
[109] Iwai I, Suda T, Tozawa F, *et al.* Stimulatory effect of Saireito on proopiomelanocortin gene expression in the rat anterior pituitary gland. Neurosci Lett 1993; 157:37-40.
[110] Sato Y, Akao T, He JX, *et al.* Glycycoumarin from Glycyrrhizae Radix acts as a potent antispasmodic through inhibition of phosphodiesterase 3. J Ethnopharmacol 2006; 105:409-14.
[111] Ikeda Y, Kaneko A, Yamamoto M, Ishige A, Sasaki H. Possible involvement of suppression of Th2 differentiation in the anti-allergic effect of Sho-seiryu-to in mice. Jpn J Pharmacol 2002; 90:328-36.
[112] Tanaka A, Ohashi Y, Kakinoki Y, *et al.* The herbal medicine shoseiryu-to inhibits allergen-induced synthesis of tumour necrosis factor alpha by peripheral blood mononuclear cells in patients with perennial allergic rhinitis. Acta Otolaryngol Suppl 1998; 538:118-25.
[113] Ikeda K, Wu DZ, Ishigaki M, Sunose H, Takasaka T. Inhibitory effects of sho-seiryu-to on acetylcholine-induced responses in nasal gland acinar cells. Am J Chin Med 1994; 22:191-6.
[114] Tohda Y, Haraguchi R, Kubo H, Muraki M, Fukuoka M, Nakajima S. Effects of saiboku-to on the survival of human eosinophils. Methods Find Exp Clin Pharmacol 1999; 21:327-30.
[115] Tamaoki J, Kondo M, Chiyotani A, Takemura H, Konno K. Effect of saiboku-to, an antiasthmatic herbal medicine, on nitric oxide generation from cultured canine airway epithelial cells. Jpn J Pharmacol 1995; 69:29-35.
[116] Sakamoto S, Kudo H, Kawasaki T, *et al.* Effects of a Chinese herbal medicine, keishi-bukuryo-gan, on the gonadal system of rats. J Ethnopharmacol 1988; 23:151-8.
[117] Mori T, Sakamoto S, Matsuda M, *et al.* Suppression of spontaneous development of uterine adenomyosis and mammary hyperplastic alveolar nodules by Chinese herbal medicines in mice. Am J Chin Med 1993; 21:263-8.
[118] Kito Y, Suzuki H. Effects of Dai-kenchu-to on spontaneous activity in the mouse small intestine. J Smooth Muscle Res 2006; 42:189-201.
[119] Kono T, Omiya Y, Hira Y, *et al.* Daikenchuto (TU-100) ameliorates colon microvascular dysfunction via endogenous adrenomedullin in Crohn's disease rat model. J Gastroenterol 2011; 46:1187-96.
[120] Nyunt AK, Takeuchi Y, Yokomuro K, Miyanaga Y. Comparative studies on the antiallergic effects of kampo medicines used for the therapy of respiratory diseases. Arerugi 1995; 44:503-12.
[121] Homma M, Minami M, Taniguchi C, *et al.* Inhibitory effects of lignans and flavonoids in saiboku-to, a herbal medicine for bronchial asthma, on the release of leukotrienes from human polymorphonuclear leukocytes. Planta Med 2000; 66:88-91.
[122] Kobayashi I, Hamasaki Y, Yamamoto S, *et al.* [Inhibitory effects of saiboku-to and compornent herbs on the production of peptide leukotrienes (LTs) and LTB4]. Arerugi 1996; 45:577-83.
[123] Yakabi K, Kurosawa S, Tamai M, *et al.* Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction. Regul Pept 2010; 161:97-105.

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The clinical use of Kampo medicines (traditional Japanese herbal treatments) for controlling cancer patients' symptoms in Japan: a national cross-sectional survey

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Abstract

Background: Kampo medicines are traditional Japanese medicines produced from medicinal plants and herbs. Even though the efficacy of Kampo medicines for controlling cancer-related symptoms is being reported, their actual nationwide clinical use has not been comprehensively investigated. We aimed to investigate physicians' recognition of Kampo medicines and their clinical use for cancer patients in the field of palliative care.

Methods: A cross-sectional self-administered anonymous questionnaire was distributed to 549 physicians working in palliative care teams at 388 core cancer treatment hospitals and 161 certified medical institutions that have palliative care units (PCUs).

Results: Valid responses were obtained from 311 physicians (response rate, 56.7%) who were evenly distributed throughout the country without significant geographical biases. Kampo medicines were prescribed for controlling cancer-related symptoms by 64.3% of the physicians. The symptoms treated with Kampo medicines were numbness/hypoesthesia (n = 99, 49.5%), constipation (n = 76, 38.0%), anorexia/weight loss (n = 72, 36%), muscle cramps (n = 71, 35.5%) and languor/fatigue (n = 64, 32.0%). Regarding open issues about prescription, 60.7% (n = 173) of the physicians raised the issue that the dosage forms need to be better devised.

Conclusions: To increase the clinical use of Kampo medicines, more evidence from clinical studies is necessary. In addition, their mechanisms of action should be clarified through laboratory studies.

Keywords: Kampo, Kampo medicine, Palliative care, Symptom management, Survey

Background

History of kampo medicine

Kampo medicines are traditional Japanese medicines produced from medicinal plants and herbs. Kampo originates from China and has been adapted to the Japanese culture [1]. Chinese herbal medicine was imported to Japan in 552 AD, after which it was uniquely developed into Japanese Kampo [2]. Traditional Chinese Medicine is deeply philosophical and ideological, while Japanese Kampo tends to be more practical and simplified, and relies little on Taoist or other Chinese philosophy [2].

Kampo medicines are currently of great interest to palliative care physicians because of their potential to alleviate the adverse side effects of cancer treatment and improve patients' quality of life.

Use of Kampo and CAM in Japan

In the past few decades, Kampo has reintegrated into modern medical practice, accompanied by a scientific reevaluation and critical examination of its relevance in conventional medicine [2,3]. Kampo has been used in addition or alternatively to conventional medicines [4]. Currently more than 70% of Japanese physicians prescribe Kampo medicines in daily clinical practices [5]. Previous survey research has reported that 76% of the general population in Japan and 50% of outpatients in

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Tokyo have used some form of CAM and that 10% of the general population and 19% of outpatients in Tokyo had used Kampo medicine prescribed by physicians within the last 12 months [6,7]. In addition, the prevalence of use of CAM by cancer patients was 44.6% in Japan [8]. Internationally, the estimates of CAM use are higher in East Asia and highest in Japan compared to the USA and European countries [9,10]. CAM is often used in palliative care settings where the goal is not cure but rather improvement in QOL [10].

To date, the Ministry of Health, Labour and Welfare (MHLW) has approved the use of 148 Kampo medicines, and the prescription of Kampo medicines is within the national health insurance system [3,11]. Although Kampo can be seen as orthodox from a historical Japanese perspective, it tends to be classified as Complementary and Alternative Medicine (CAM) according to Western conventions. The main reason for this is the lack of scientific evidence of its efficacy and the limited knowledge and spread of this therapy in other regions, especially outside of East Asia.

However, clinical studies of Kampo have been conducted in Japan, and its efficacy has been reported in research papers. For example, a randomized control trial demonstrated that the Kampo medicine *Rikkunshito* exerted greater effects in alleviating gastrointestinal symptoms than cisapride (a gastroprokinetic agent) [12]. The efficacy of *Rikkunshito* against non-ulcer dyspepsia (NUD) [13,14], gastrointestinal symptoms after gastrectomy (surgical NUD) [15], functional dyspepsia [16,17], and nausea and vomiting caused by selective serotonin reuptake inhibitors [18] has also been reported. Also, the Japanese Society for Oriental Medicine has compiled comprehensive data on randomized controlled trials of Kampo medicine in Japan, published as "Evidence Reports of Kampo Treatment" (EKAT) [19]. In addition to clinical trials, the potential mechanisms of action of Kampo medicines are also starting to be reported [20].

As described above, there is increasing evidence of the efficacy of Kampo medicines and increasing attention has been given to their clinical application. However, there has been no comprehensive investigation of the use of Kampo medicines in cancer treatment. Therefore, we conducted a nationwide survey of the current use of Kampo medicines for cancer-related treatment and of physicians' attitudes toward using Kampo medicines in Japan.

Methods

Study sample and data collection

The survey was carried out between January and March of 2011, by mailing a self-administered anonymous questionnaire to 549 palliative care physicians who administer chemotherapy to cancer patients or who are involved in their terminal care. The palliative care teams in 388 core cancer treatment hospitals and 161 palliative care units (PCUs) within medical institutions were selected because they represent palliative care practice in Japan. This included all core cancer treatment hospitals and PCUs in Japan as of February 2011. Core cancer treatment hospitals are the medical facilities specified by the MHLW to provide high-quality expert care for cancer patients. These facilities are established within each prefecture in Japan, according to the principles set forth in the Cancer Control Act promulgated in April 2007. The contact information of subjects was obtained from a web site of the Cancer Control Information Center, National Cancer Center [21].

We did not specifically include general internists or surgeons who are not in charge of palliative care as subjects of the survey. This is because the certification system for the palliative care specialist is still immature in Japan and the attending physicians of palliative care teams and PCUs are often internists or surgeons.

Questionnaire development

An eight-page, 18-item questionnaire was designed in Japanese. It covered four categories: (1) status of cancer treatment and use of Kampo medicines, (2) cancer cachexia and utilization of Kampo medicines (data not shown), (3) adverse side effects of anti-cancer drugs and utilization of Kampo medicines, and (4) background variables. Although the questionnaire was not formally validated, the questionnaire and its items were designed and formulated based upon the expert opinions of specialists from palliative care, medical oncology, Kampo medicine, and biological statistics, and also from literature reviews. It was finalized after testing several samples.

Ethical considerations

We conducted this research in compliance with the Helsinki Declaration. We had requested an ethical review of this research from the ethical review committee of the National Cancer Center prior to commencement. However, since this research involves neither patients' data nor intervention, the committee judged that this research should not be subjected to any Japanese medical research guidelines. Accordingly, the research was exempt from the requirement for formal ethical approval.

To ensure that informed consent was obtained, the questionnaire was sent to the physicians with a leaflet explaining the survey's objectives and that (1) each subject was free to decide whether or not to answer the questions; (2) the collected data will be processed and analyzed anonymously; and (3) the data will be securely archived by the Research Secretariat. Consent was implied through the return of a completed questionnaire.

Data analysis

The collected data were entered into an electronic database and analyzed using SPSS (IBM, New York, USA). Chi-squared tests (p value < 0.050) were conducted to compare the frequency distributions of two cross-tabulations. The first was physicians in the palliative care teams at the core cancer treatment hospitals compared with physicians in the PCUs. The second was the palliative care specialists certified by the Japan Society of Palliative Medicine (JSPM) compared with non-specialists.

Results and discussion

Of the 549 questionnaires distributed, 311 valid responses were collected for analysis (response rate, 56.7%). Responses were obtained from 226 physicians (response rate, 58.2%) at core cancer treatment hospitals (palliative care team physicians) and 79 physicians (response rate, 49.1%) from PCUs (PCU physicians). With the moderate rate of valid responses (56.7%), the respondents were well-distributed throughout the country, without significant geographical biases. Table 1 shows the response rates and the respondents' background characteristics. Two hundred thirty seven respondents (77.9%) were aged between 40 and 59 years. Two hundred seventy three respondents (90.1%) were male, and 128 respondents (41.2%) were JSPM-authorized palliative care specialists (including provisional medical advisors).

Difficult to treat cancer-related symptoms

Physicians were asked to identify which of the 23 common cancer-related symptoms that they find difficult to treat (Table 2). More than 50% of the physicians identified *numbness/hypoesthesia* (n = 240, 77.2%), *languor/fatigue* (n = 225, 72.3%), *delirium* (N = 170, 54.7%), and *taste alteration* (n = 166, 53.4%). In comparison with the PCU physicians, more palliative care team physicians identified *taste alteration* (p = 0.029), *nausea/vomiting (during chemotherapy)* (p = 0.000), and *constipation (caused by opioid use)* (p = 0.038). More of the PCU physicians, on the other hand, reported having difficulty treating *adjustment disorder* (p = 0.014). In addition, the symptoms of *taste alteration* (p = 0.050), *dysphagia/deglutition disorder* (p = 0.036) and *muscle weakness* (p = 0.047) were

Table 1 Respondents' background characteristics

Respondents (n = 311)			Average ± SD	Minimum value	Maximum value		
Age			49 ± 8	28	75		
Years of experience			23 ± 8	4	50		
			Responses	%			
Institution (n = 549) *							
Core cancer treatment hospital (n = 388)			226	58.2			
Palliative Care Unit in medical institution (n = 161)			79	49.1			
			n	%			
Age group							
20–29 years			1	0.3			
30–39 years			39	12.8			
40–49 years			119	39.1			
50–59 years			118	38.8			
≥ 60 years			27	8.9			
Sex							
Male			273	90.1			
Female			30	9.9			
Palliative Care Specialists certified by JSPM**							
Specialists (including provisional medical advisors)			128	41.2			
Non-specialists			183	58.8			
Region***	Hokkaido–Tohoku	Kanto	Chubu	Kinki	Chugoku	Shikoku	Kyushu–Okinawa
Number of questionnaires distributed	79	116	92	91	47	27	97
Number of responses	26	43	41	33	25	11	37
Response rate (%)	32.9	37.1	44.6	36.3	53.2	40.7	38.1

*Six responses had missing institution data, and ***95 responses had missing region data.
** JSPM: Japan Society for Palliative Medicine.

Table 2 Difficult to treat cancer-related symptoms identified by physicians

Symptoms	All physicians (n = 311)		Palliative care teams (n = 226)		PCUs (n = 79)		p-value	Specialists (n = 128)		Non-specialists (n = 183)		p-value
	frequency	%	frequency	%	frequency	%		frequency	%	frequency	%	
Numbness/Hypesthesia	240	77.2	180	79.6	55	69.6	0.165	99	77.3	141	77.0	1.000
Languor/Fatigue	225	72.3	161	71.2	61	77.2	0.276	99	77.3	126	68.9	0.122
Delirium	170	54.7	119	52.7	48	60.8	0.447	73	57.0	97	53.0	0.490
Taste alteration	166	53.4	124	54.9	42	53.2	0.029	77	60.2	89	48.6	0.050
Edema (Local edema/Anasarca)	150	48.2	109	48.2	39	49.4	0.821	59	46.1	91	49.7	0.565
Pain	146	46.9	113	50.0	31	39.2	0.226	55	43.0	91	49.7	0.250
Anorexia/Weight loss	140	45.0	109	48.2	30	38.0	0.108	64	50.0	76	41.5	0.165
Abdominal discomfort	131	42.1	98	43.4	31	39.2	0.735	55	43.0	76	41.5	0.816
Stomatitis/Xerostomia	122	39.2	89	39.4	33	41.8	0.141	54	42.2	68	37.2	0.409
Depression	116	37.3	86	38.1	30	38.0	0.175	41	32.0	75	41.0	0.122
Adjustment disorder	113	36.3	73	32.3	39	49.4	0.014	47	36.7	66	36.1	1.000
Dyspnea/Breathlessness	113	36.3	77	34.1	35	44.3	0.162	48	37.5	65	35.5	0.811
Nausea/Vomiting (other)	101	32.5	75	33.2	24	30.4	0.893	38	29.7	63	34.4	0.392
Dysphagia/Deglutition disorder	100	32.2	68	30.1	31	39.2	0.281	50	39.1	50	27.3	0.036
Sleep disorder/Insomnia	93	29.9	69	30.5	23	29.1	0.796	42	32.8	51	27.9	0.379
Constipation (caused by opioid use)	84	27.0	69	30.5	15	19.0	0.038	34	26.6	50	27.3	0.898
Nausea/Vomiting (during chemotherapy)	76	24.4	71	31.4	5	6.3	0.000	27	21.1	49	26.8	0.284
Muscle weakness	65	20.9	46	20.4	19	24.1	0.346	34	26.6	31	16.9	0.047
Nausea/Vomiting (caused by opioid use)	61	19.6	51	22.6	10	12.7	0.690	24	18.8	37	20.2	0.774
Constipation (not caused by opioid use)	59	19.0	47	20.8	11	13.9	0.377	28	21.9	31	16.9	0.305
Muscle cramp	42	13.5	31	13.7	11	13.9	0.741	23	18.0	19	10.4	0.064
Diarrhea	40	12.9	34	15.0	6	7.6	0.136	16	12.5	24	13.1	1.000
Anemia	29	9.3	24	10.6	5	6.3	0.344	16	12.5	13	7.1	0.177
Others	11	3.5	6	2.7	5	6.3	0.325	4	3.1	7	3.8	0.770

Multiple answers allowed, p-value based on Chi-square test.

identified as being difficult to treat more often by the palliative care specialists than the non-specialists.

Numbness is a neuropathic symptom that frequently occurs as an adverse side effect of chemotherapy. It has been reported to account for 58% of all neurological symptoms experienced by cancer patients [22]. Fatigue is the most common cancer symptom [23], and was reported by 66% of patients in a previous study [22]. The prevalence of delirium is 25–40% (85–88% in the terminal stage of cancer) [24-26], and the prevalence of taste alteration is 36–75% among patients receiving chemotherapy [27]. Thus, it was shown in the present survey that the symptoms palliative care physicians have difficulty managing in Japan are those frequently seen in cancer patients.

We also found that the palliative care team physicians confront *taste alteration* (p = 0.029), *nausea/vomiting during chemotherapy* (p = 0.000) and *constipation during opioid use* (0.038) more often than the PCU physicians (Table 2). These facts suggest that the palliative care teams are often in charge of patients receiving chemotherapy, while PCUs are more frequently dealing with psychiatric symptoms than the adverse side effects of chemotherapy.

Prescription of Kampo medicines

Kampo medicines were being prescribed by 64.3% (n = 200) of the physicians to alleviate the cancer patients' symptoms. Kampo medicines were prescribed to control *numbness/hypoesthesia* (n = 99, 49.5%), *constipation (not caused by opioid use)* (n = 76, 38%), *anorexia/weight loss* (n = 72, 36%), *muscle cramps* (n = 71, 35.5%), and *languor/fatigue* (n = 64, 32%) by more than 30% of the physicians (Table 3). The palliative care team physicians prescribed Kampo medicines for *numbness/hypoesthesia* (p = 0.000), *anorexia/weight loss* (p = 0.046), *pain* (p = 0.020), and *nausea/vomiting during chemotherapy* (p = 0.016), more frequently than the PCU physicians. This difference may arise because the palliative care teams more often examine patients who are under chemotherapy than the PCUs, and thus they pay more

Table 3 Symptoms for which Kampo medicines were prescribed

Symptoms	All physicians (n = 200)		Palliative care teams (n = 149)		PCUs (n = 46)		p-value
	frequency	%	frequency	%	frequency	%	
Numbness/Hypesthesia	99	49.5	86	57.7	12	26.1	0.000
Constipation (not caused by opioid use)	76	38	56	37.6	20	43.5	0.182
Anorexia/Weight loss	72	36	60	40.3	12	26.1	0.046
Muscle cramp	71	35.5	54	36.2	17	37.0	0.279
Languor/Fatigue	64	32	49	32.9	14	30.4	0.818
Constipation (caused by opioid use)	48	24	37	24.8	11	23.9	0.490
Abdominal discomfort	46	23	29	19.5	16	34.8	0.088
Diarrhea	45	22.5	39	26.2	5	10.9	0.090
Delirium	40	20	27	18.1	13	28.3	0.155
Pain	38	19	35	23.5	3	6.5	0.020
Edema (Local edema/Anasarca)	31	15.5	25	16.8	6	13.0	0.546
Nausea/Vomiting (other)	27	13.5	22	14.8	5	10.9	0.566
Nausea/Vomiting (during chemotherapy)	22	11	22	14.8	0	0.0	0.016
Stomatitis/Xerostomia	21	10.5	19	12.8	2	4.3	0.216
Taste alteration	20	10	17	11.4	3	6.5	0.409
Depression	20	10	17	11.4	3	6.5	0.409
Nausea/Vomiting (caused by opioid use)	17	8.5	16	10.7	1	2.2	0.129
Adjustment disorder	15	7.5	12	8.1	3	6.5	0.846
Sleep disorder/Insomnia	14	7	10	6.7	4	8.7	0.823
Others	13	6.5	6	4.0	6	13.0	0.055
Anemia	11	5.5	9	6.0	2	4.3	0.805
Dysphagia/Deglutition disorder	10	5	9	6.0	1	2.2	0.581
Dyspnea/Breathlessness	6	3	5	3.4	1	2.2	1.000
Muscle weakness	3	1.5	3	2.0	0	0.0	0.614

Multiple answers allowed, p-value based on Chai-square test.

attention than the PCUs to the necessity of controlling the adverse side effects of chemotherapy. Also, PCU patients have more difficulty taking Kampo medicines than the general hospital patients under the palliative care teams. The frequency of prescribing Kampo medicines did not vary significantly across the symptoms between the palliative care specialists and non-specialists.

Reasons for prescription

More than 60% of the physicians prescribed Kampo medicines for the following reasons: 'the drug therapy options are greater' (n = 144, 72%), 'ineffectiveness of other treatments' (n = 129, 64.5%), and 'unavailability of other appropriate treatments' (n = 127, 63.5%). Although 'patient demand' was the least frequent reason (n = 46, 23%), palliative care specialists were more attentive to patients' demands than non-specialists (n = 28, 37.3%, p = 0.000).

Variety and frequency of prescriptions

Eight Kampo medicines were selected from the literature reviews to investigate frequency of prescription. Table 4 shows the composition of each Kampo medicine [28-30]. *Daikenchuto* was the most frequently prescribed (n = 140, 70%) among eight major Kampo medicines (Table 5). This is probably because the efficacy of *Daikenchuto* for the treatment of gastrointestinal symptoms is currently being tested in clinical trials in Japan and the United States. A tolerability and efficacy phase II study of *Daikenchuto* for the treatment of postoperative ileus has been already completed in the United States [31]. This might encourage its prescription by physicians. The palliative care team physicans prescribed *Goshajinkigan* (p = 0.000), *Rikkunshito* (p = 0.001), *Hochuekkito* (p = 0.011), *Juzentaihoto* (p = 0.001), and *Hangeshashinto* (p = 0.000) more frequently than PCU physicians, while there were no significant differences in the medicines prescribed between the palliative care specialists and non-specialists.

Physician-recognized effectiveness

We investigated the physician-recognized effectiveness of eight Kampo medicines. Two symptoms from each Kampo medicine's package insert were listed and the physicians were asked to indicate whether they believed the medicine effectively treated them (Table 6). More than 50% of the

Table 4 Composition of Kampo medicines

Kampo Medicine	Ingredients (crude drugs)									
Hangeshashinto	Pinelliae Tuber	Scutellariae Radix	Zingiberis Processum Rhizoma	Glycyrrhizae Radix	Zizyphi Fructus	Ginseng Radix	Coptidis Rhizoma			
Hochuekkito	Astragali Radix	Atractylodis lanceae Rhizoma	Ginseng Radix	Angelicae Radix	Bupleuri Radix	Zizyphi Fructus	Aurantii Nobilis Pericarpium	Glycyrrhizae Radix	Cimicifugae Rhizoma	Zingiberis Rhizoma
Rikkunshito	Atractylodis lanceae Rhizoma	Ginseng Radix	Pinelliae Tuber	Poria	Zizyphi Fructus	Aurantii Nobilis Pericarpium	Glycyrrhizae Radix	Zingiberis Rhizoma		
Juzentaihoto	Astragali Radix	Cinnamomi Cortex	Rehmanniae Radix	Paeoniae Radix	Cnidii Rhizoma	Atractylodis lanceae Rhizoma	Angelicae Radix	Ginseng Radix	Poria	Glycyrrhizae Radix
Yokukansan	Atractylodis lanceae Rhizoma	Poria	Cnidii Rhizoma	Uncariae Uncis cum Ramulus	Angelicae Radix	Bupleuri Radix	Glycyrrhizae Radix			
Shakuyakukanzoto	Glycyrrhizae Radix	Paeoniae Radix								
Daikenchuto	Zingiberis Processum Rhizoma	Ginseng Radix	Zanthoxyli Fructus							
Goshajinkigan	Rehmanniae Radix	Achyranthis Radix	Corni Fructus	Dioscoreae Rhizoma	Plantaginis Semen	Alismatis Rhizoma	Poria	Moutan Cortex	Cinnamomi Cortex	Processi Aconiti Radix

Ingredients of each Kampo medicine were based on the package inserts of Tsumura products [28].
Scientific names of ingredients were based on Metabolomics.jp [29] and The Japanese Pharmacopeia Fifteenth edition [30].

Table 5 The Kampo medicines prescribed by the physicians

Kampo medicine	All physicians (n = 200)		Palliative care teams (n = 149)		PCUs (n = 46)		p-value
	frequency	%	frequency	%	frequency	%	
Daikenchuto	140	70.0	109	73.2	29	63.0	0.124
Goshajinkigan	100	50.0	89	59.7	11	23.9	0.000
Rikkunshito	97	48.5	82	55.0	15	32.6	0.001
Shakuyakukanzoto	96	48.0	76	51.0	20	43.5	0.069
Hochuekkito	90	45.0	76	51.0	13	28.3	0.011
Juzentaihoto	84	42.0	73	49.0	11	23.9	0.001
Yokukansan	61	30.5	45	30.2	16	34.8	0.253
Hangeshashinto	54	27.0	51	34.2	3	6.5	0.000
Others	24	12.0	20	13.4	4	8.7	0.457

Multiple answers allowed, p-value based on Chi-square test.

physicians recognized the effectiveness of *Hangeshashinto* against *diarrhea caused by chemotherapy* (n = 31, 53.4%), of *Hochuekkito* and *Juzentaihoto* against *fatigue* (n = 54, 56.3% and n = 50, 56.8% respectively), of *Rikkunshito* against *anorexia* (n = 46, 50%), of *Yokukansan* against *delirium* (n = 38, 63.3%), of *Shakuyakukanzoto* against *leg cramps* (n = 79, 82.3%), and of *Daikenchuto* against *ileus* (n = 101, 78.9%) and *opioid-caused constipation and abdominal pain* (n = 62, 53.9%). There was no significant difference in the medicines recognized as effective between the palliative care team and PCU physicians, while the palliative care specialists seemed to be more aware of the effectiveness of *Rikkunshito* against *nausea* than non-specialists (p = 0.012) (Table 6). These results suggest that there is consensus among palliative care physicians regarding the effectiveness of particular Kampo medicines against particular symptoms.

Prescription considerations

In the questionnaire, the physicians were asked, "What are the important considerations when selecting a Kampo medicine for prescription?". More than 80% of the physicians recognized the importance of 'symptom-alleviating effects (alleviation of adverse side effects) (n = 173, 93%)', 'alleviation of symptoms that reduce QOL in the terminal stage of cancer' (n = 162, 87.6%), 'low incidence of adverse side effects' (n = 157, 84.9%) and 'easy

Table 6 Physician-recognized effectiveness of Kampo medicines

Kampo medicine	Symptoms	Recognized as effective						p-value
		All physicians		Specialists		Non-specialists		
		frequency/total	%	frequency/total	%	frequency/total	%	
Hangeshashinto	Diarrhea caused by chemotherapy	31/58	53.4	10/22	45.5	21/36	58.3	0.420
	Nausea	10/45	22.2	3/21	14.3	7/24	29.2	0.296
Hochuekkito	Anorexia	44/90	48.9	14/36	38.9	30/54	55.6	0.137
	Fatigue	54/96	56.3	19/39	48.7	35/57	61.4	0.295
Rikkunshito	Nausea	36/82	43.9	9/34	26.5	27/48	56.3	0.012
	Anorexia	46/92	50.0	18/40	45.0	28/52	53.8	0.528
Juzentaihoto	Fatigue	50/88	56.8	17/33	51.5	33/55	60.0	0.508
	AE caused by chemotherapy or radiotherapy	27/58	46.6	7/22	31.8	20/36	55.6	0.106
Yokukansan	Delirium	38/60	63.3	18/26	69.2	20/34	58.8	0.433
	Anxiety	15/50	30.0	6/23	26.1	9/27	33.3	0.758
Shakuyakukanzoto	Leg cramps	79/96	82.3	36/43	83.7	43/53	81.1	0.794
	Abdominal pain	20/57	35.1	11/25	44.0	9/32	28.1	0.268
Daikenchuto	Ileus	101/128	78.9	35/48	72.9	66/80	82.5	0.263
	Opioid-caused constipation and abdominal pain	62/115	53.9	22/47	46.8	40/68	58.8	0.254
Goshajinkigan	Numbness of hands and feet	47/107	43.9	18/39	46.2	29/68	42.6	0.840
	Nocturia	13/60	21.7	4/26	15.4	9/34	26.5	0.358

Multiple answers allowed, p-value based on Chi-square test.

Table 7 Open issues about prescribing Kampo medicine (n = 285)

Issue	frequency	%
The dose and dosage forms need to be better devised for simpler application	173	60.7
No evidence of efficacy from placebo-controlled studies	109	38.2
Action mechanism of Kampo medicine is not yet elucidated	97	34.0
No opportunity to learn about Kampo medicines	90	31.6
Relatively weak effect	79	27.7
Drug interaction is uncertain	66	23.2
Production of effect is slow	56	19.6
Others	25	8.8
There are no issues	12	4.2

Multiple answers allowed.

to combine with other drugs' (n = 149, 80.5%). The palliative care specialists tended to place more importance than the non-specialists on 'patient demand' (p = 0.050).

Open issues for prescription

The questionnaire also asked the physicians to identify any open issues regarding the prescription of Kampo medicines (Table 7), revealing that 60.7% (n = 173) of the physicians were concerned that the dose and dosage forms need to be better devised for simpler administration. Kampo medicines are commonly prepared in granule form or as decoctions, and their administration method is nauseating for some patients. This issue may be related to the observation that "patient demand" was chosen least frequently as the reason for prescription. In the clinical field of palliative care, Kampo medicines are often mixed in a jelly for patients who have dysphagia. For future prescriptions, the administration forms need to be better devised from an adherence perspective. The second most frequently identified issue was the lack of scientific evidence for their efficacy, with 38.2% (n = 109) of the physicians highlighting the absence of evidence from placebo-controlled trails. Watanabe *et al.* [3] recently reported a summary of 135 peer-reviewed Kampo trials published between 1988 and 2007. According to their report, 106 trials were RCTs, and only 22 were placebo-controlled trials. In two-thirds of the trials, the sample size was less than 100 patients, and only 35 trials were published in English and the rest were in Japanese. Watanabe *et al.* [3] concluded that the overall quality of the research was low.

Conclusions

We conducted a nationwide survey of 311 physicians working in palliative care teams at core cancer treatment hospitals and PCUs within medical facilities. Kampo medicines were prescribed by a high proportion (n = 200, 64.3%) of the palliative care physicians and were expected to provide valid means of controlling the cancer patients' symptoms or the adverse side effects of chemotherapy. Palliative care physicians appear to be aware of the effectiveness of Kampo medicines. However, they prescribe Kampo medicines only to a limited extent because of the lack of evidence for their efficacy. Hence, we believe that the collection of more evidence from clinical studies is desirable in Japan.

Abbreviations

MHLW: Ministry of health labour and welfare; CAM: Complementary and alternative medicine; PCUs: Palliative care units.

Competing interests

The authors declare that they have no competing interests. The authors were free to interpret the data according to a strict scientific rationale.

Authors' contributions

YU conceived the study idea and SI and TY contributed to the study design and concept. YU distributed and collected the questionnaires. SI, TY and TM processed and analyzed the data. SI and TM wrote the initial manuscript. All authors interpreted the data and approved the final manuscript.

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References

1. Yu F, Takahashi T, Moriya J, Kawaura K, Yamakawa J, Kusaka K, Itoh T, Morimoto S, Yamaguchi N, Kanda T: **Traditional Chinese medicine and Kampo: a review from the distant past for the future.** *J Int Med Res* 2006, **34**:231–239.
2. Terasawa K: **Evidence-based reconstruction of Kampo medicine: Part I –Is Kampo CAM?** *Evid Based Complement Altern Med* 2004, **1**:11–16.

3. Watanabe K, Matsuura K, Gao P, Hottenbacher L, Tokunaga H, Nishimura K, Imazu Y, Reissenweber H, Witt CM: **Traditional Japanese Kampo medicine: Clinical research between modernity and traditional medicine-the state of research and methodological suggestions for the future.** *Evid Based Complement Altern Med* 2011, **2011**:1–19.
4. Nishimura K, Plotnikoff GA, Watanabe K: **Kampo medicine as an integrative medicine in Japan.** *Jpn Med Assoc J* 2009, **52**(3):147–149.
5. Watanabe K: *General Practitioner Should Use Kampo Medicine.* http://mric.tanaka.md/2008/10/22/_vol_148.html.
6. Yamashita H, Tsukayama H, Sugishita C: **Popularity of complementary and alternative medicine in Japan: a telephone survey.** *Complement Ther Med* 2002, **10**(2):84–93.
7. Hori S, Mihaylov I, Vasconcelos JC, McCoubrie M: **Patterns of complementary and alternative medicine use amongst outpatients in Tokyo, Japan.** *BMC Complement Altern Med* 2008, **8**:14.
8. Hyodo I, Amano N, Eguchi K, Narabayashi M, Imanishi J, Hirai M, Nakano T, Takashima S: **Nationwide survey on complementary and alternative medicine in cancer patients in Japan.** *J Clin Oncol* 2005, **23**(12):2645–2654.
9. Harris PE, Cooper KL, Relton C, Thomas KJ: **Prevalence of complementary and alternative medicine (CAM) use by the general population: a systematic review and update.** *Int J Clin Pract* 2012, **66**:915–916.
10. Molassiotis A, Fernadez-Ortega P, Pud D, Ozden G, Scott JA, Panteli V, Margulies A, Browall M, Magri M, Selvekerova S, Madsen E, Milovics L, Bruyns I, Gudmundsdottir G, Hummerston S, Ahmad AMA, Platin N, Kearney N, Patiraki E: **Use of complementary and alternative medicine in cancer patients: a European survey.** *Ann Oncol* 2005, **16**:655–663.
11. Arai I: *The current situation of the Japanese medical plants industry and its significance for the pharmaceutical industry.* http://ndais.coa.gov.tw/htmlarea_file/web_articles/ndais/1354/980108_1.pdf.
12. Miyoshi A, Yachi A, Masamune O, Ishikawa M, Fukutomi H, Niwa H, Matsuo Y, Mori H, Harasawa S: **Clinical evaluation of Rikkunshito (TJ-43 TSUMURA & Co.) for indeterminate digestive complaints including chronic gastritis, etc.. Multi-institutional study with reference drug of Cisapride.** *Prog Med* 1991, **11**:1605–3. in Japanese.
13. Tatsuta M, Iishi H: **Effect of treatment with liu-jun-zi-tang (TJ-43) on gastric emptying and gastrointestinal symptoms in dyspeptic patients.** *Aliment Pharmacol Ther* 1993, **7**:459–462.
14. Harasawa S, Miyoshi A, Miwa T, Masamune O, Matsuo Y, Nakazawa S, Suyama T, Hayakawa A, Nakajima M: **Clinical test of ataxia-type upper abdominal disorder (dysmotility-like dyspepsia) after multiple facilities cooperated marketing of Kampo medicine TJ-43 Rikkunnshitou. Examination by double blind experiment group comparison.** *J Clin Exp Med (IGAKKU NO AYUMI)* 1998, **187**:207–229. in Japanese.
15. Gochi A, Hirose S, Sato K, Hiramatsu S, Asakura A, Tokuoka H, Matsuno T, Kamikawa Y, Orita K: **The Effect of Hange-shashin-to and Rikkunshi-to against the digestive symptoms after gastrectomy.** *Jpn J Gastroenterol Surg* 1995, **28**:961–965. in Japanese.
16. Nakajima O, En S: **Comparative analysis of the Kampo medicine Rikkunshito's clinical efficacy on patients with functional dyspepsia.** *Jpn J Med Pharmacol Sci* 2008, **59**:235–240. in Japanese.
17. Endo G: **Analysis of the Rikkunshito efficacy on patients with functional dyspepsia.** *Jpn J Med Pharmacol Sci* 2008, **60**:547–552. in Japanese.
18. Oka T, Tamagawa Y, Hayashida S, Kaneda Y, Kodama N, Tsuji S: **Rikkunshi-to attenuates adverse gastrointestinal symptoms induced by fluvoxamine.** *Biopsychosoc Med* 2007, **1**:21.
19. Okabe T, Tsutani K: *Evidence Reports of Kampo Treatment (EKAT) Appendix 2011.* http://www.jsom.or.jp/medical/ebm/ere/pdf/EKATE_Appendix_2011.pdf.
20. Uezono Y, Miyano K, Sudo Y, Suzuki M, Shiraishi S, Terawaki K: **A review of traditional japanese medicines and their potential mechanism of action.** *Curr Pharm Des* 2012, **18**:1–15. In Press.
21. Cancer Control Information Center, National Cancer Center: *Cancer Information Service.* http://ganjoho.jp/public/index.html.
22. Kautio AL, Haanpää M, Kautiainen H, Kalso E, Saarto T: **Burden of chemotherapy-induced neuropathy-a cross-sectional study.** *Support Care Cancer* 2010, **19**:1991–1996.
23. Cella D, Lai JS, Chang CH, Peterman A, Slavin M: **Fatigue in cancer patients compared with fatigue in the general United States population.** *Cancer* 2002, **94**(2):528–538.
24. Breitbart W, Bruera E, Chochinov H, Lynch M: **Neuropsychiatric syndromes and psychological symptoms in patients with advanced cancer.** *J Pain Symptom Manage* 1995, **10**:131–141.
25. Massie MJ, Holland JC, Glass E: **Delirium in terminally ill cancer patients.** *Am J Psychiatry* 1983, **140**:1048–1050.
26. Lawlor PG, Gagnon B, Mancini IL, Pereira JL, Hanson J, Suarez-Almazor ME, Bruera ED: **Occurrence, causes and outcomes of delirium in patients with advanced cancer.** *Arch Intern Med* 2000, **160**:786–794.
27. Ravasco P: **Aspects of taste and compliance in patients with cancer.** *Eur J Oncol Nurs* 2005, **9**:84–91.
28. Tsumura & Co: *Product Information.* http://www.tsumura.co.jp/password/m_square/products/ichiran/ (in Japanese).
29. Arita M: *Crude Drug List.* http://metabolomics.jp/wiki/Persist:CrudeDrugList.
30. *The Japanese pharmacopeia fifteenth edition.* http://jpdb.nihs.go.jp/jp15/ (in Japanese).
31. *A tolerability and efficacy study of TU-100 for the treatment of postoperative ileus.* http://clinicaltrials.gov/ct2/show/NCT00266461?term=Daikenchuto+%28TU-100%29&rank=3.

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REVIEW ARTICLE

The recent progress in research on effects of anesthetics and analgesics on G protein-coupled receptors

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Abstract The exact mechanisms of action behind anesthetics and analgesics are still unclear. Much attention was focused on ion channels in the central nervous system as targets for anesthetics and analgesics in the 1980s. During the 1990s, major advances were made in our understanding of the physiology and pharmacology of G protein coupled receptor (GPCR) signaling. Thus, several lines of studies have shown that G protein coupled receptors (GPCRs) are one of the targets for anesthetics and analgesics and especially, that some of them inhibit the functions of GPCRs, i.e,, muscarinic receptors and substance P receptors. However, these studies had been focused on only G_q coupled receptors. There has been little work on G_s- and G_i-coupled receptors. In the last decade, a new assay system, using chimera $G_{i/o}$-coupled receptor fused to Gq_{i5}, has been established and the effects of anesthetics and analgesics on the function of G_i-coupled receptors is now more easily studied. This review highlights the recent progress of the studies regarding the effects of anesthetics and analgesics on GPCRs.

Keywords Anesthetics · Analgesics · G protein-coupled receptor

Introduction

In the 1990s, the effects of anesthetics on voltage- and ligand-gated ion channels have been the focus of several studies [1–4]. However, the mechanisms of anesthetics and analgesics actions are still not known well. The G protein coupled receptors (GPCRs) are not only the largest protein family in the human genome but are also the single biggest target for many drugs (Fig. 1; Table 1). Recent research about GPCRs is therefore growing at a fast pace and the range of techniques that can be applied to GPCRs is vast and continues to grow in our understanding of the physiology and pharmacology of G protein coupled receptor (GPCR) signaling. Further studies have shown that GPCRs are targets for anesthetics [5]. As compared with ion ligand-gated ion channels, less is known about the mechanisms of action of anesthetics on GPCRs. In this review, we present the recent progress of the research on the effects of anesthetics and analgesics on GPCRs.

The effect of anesthetics and analgesics on G_q protein coupled receptors

The main focus of GPCR anesthetics and analgesics research has often been concentrated on G_q-coupled receptors (Tables 2, 3). Because G_q coupled receptors leads to intracellular Ca^{2+} elevation, the effects of anesthetics and analgesics on G_q coupled receptors have been well studied using the *Xenopus* oocyte expression system (Fig. 2). The *Xenopus* oocyte expression system has been used to study a multiplicity of brain receptors with pharmacological properties that mimic those of native brain receptors [2]. Stimulation of G_q coupled receptors results in activation of Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes [6–9]; stimulation of G_q coupled receptors leads to G protein-dependent activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol. The IP_3 causes the release of Ca^{2+} from the endoplasmic

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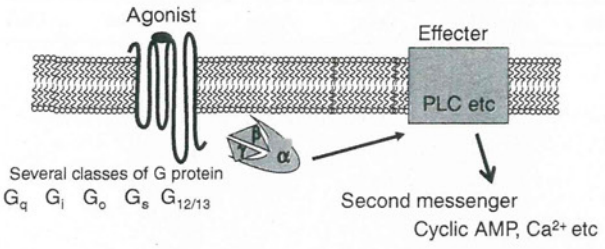

Fig. 1 Intracellular signaling of G protein coupled receptor

Table 1 Signaling of G protein coupled receptor

G protein	G_q	$G_{i/o}$	G_s
Effecter	PLC↑	Adenylate cyclase↓	Adenylate cyclase↑
Second messenger	IP_3 ↑ DAG↑	cAMP↓	cAMP↑
Intracellar reaction	PKC↑ Ca^{2+}↑	PKA↓	PKA↑
Receptor	M_1	M_2	β-Adrenergic
	M_3	μ Opioid	
	Substance P		
	Orexin 1		
	$5HT_{2A}$		
	mGluR1		
	mGluR1		

Table 2 The effects of volatile anesthetics on Gq coupled receptor function

	Halothane	Isoflurane	Enflurane	Desflurane	Sevoflurane
M_1	↓	→		↓↑	↓
M_3	↓	↓		→	↓
$5HT_{2A}$	↓				
$5HT_{2C}$		↓	↓		
mGluR1	→				
mGluR5	↓				
Substance P	↓	↓	↓		↓
Orexin 1	↓	↓	↓		

reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. This system has been well characterized, and has proven useful for studying the effects of anesthetics and analgesics on G_q coupled receptors.

Muscarinic acetylcholine receptors

In G_q coupled receptors, muscarinic acetylcholine receptors (MRs) have been paid much attention to as the target of the anesthetics and analgesics. This is because MRs are involved in various neuronal functions in the central nervous system (CNS) and the autonomic nervous system [10]. Cholinergic antagonism interferes with learning behavior, whereas cholinesterase inhibitors enhance learning [11]. Furthermore, the inhibition of MRs lead to sedation or non-rapid eye movement sleep [12]. The therapeutic potential of muscarinic antagonists is compromised by several effects on the autonomic nervous system, including dry mouth, tachycardia, constipation, urinary retention, and pupillary dilation [13]. Recent molecular cloning studies have revealed the existence of five subtypes of MRs (M1R-M5R) [14, 15]. Using pharmacological techniques, many of the muscarinic responses in peripheral tissues have been studied thoroughly. However, relatively little is known about the functional roles of individual subtypes of MRs in the CNS. Recent studies of their anatomic distribution have been used to predict their functions in the CNS. For example, cortical and hippocampal M1R are involved in memory and learning [16].

To date, several investigators have studied the effects of anesthetics on MRs. Anthony et al. [17] reported that chloroform, enflurane, isoflurane and halothane increased the affinity of [^{3}H]methylscopolamine([^{3}H]MS) binding, but did not affect the number of [^{3}H]MS binding sites in the rat brainstem. Isoflurane inhibits muscarinic receptor-evoked cyclic GMP production in cultured bovine adrenal medullary cells, suggesting that isoflurane inhibits M1R [18]. Lin et al. [19] showed that enflurane inhibits the function of mouse and human brain phosphatidylinositol-linked MRs expressed in *Xenopus* oocytes.

There have been several reports on the effects of volatile anesthetics on recombinant MRs using the *Xenopus* oocyte expression system. Halothane inhibits signaling via M1R expressed in *Xenopus* oocytes [6–12]. Desflurane has a biphasic effect on M1R signaling, enhancing it at lower concentrations, but depressing it at higher concentrations and a similar, although not significant, trend was observed with M3R signaling [20]. Isoflurane has no effect on M1R signaling, but inhibits M3R signaling [20, 21]. Sevoflurane depresses the function of M1R and M3R signaling in a dose-dependent manner [22]. Similar to its known effect on M1R signaling, halothane also depresses M3R function dose-dependently [22].

There are several reports on the inhibitory effects of intravenous anesthetics, ketamine, propofol, thiopental, alphaxalone and an α_2-adrenoceptor agonist, dexmedetomidine. Durieux [23] reported that ketamine profoundly inhibits muscarinic signaling. Nagase et al. [24] reported that propofol inhibits M1R-mediated signal transduction at the receptor site or the site of interaction between the receptor and associated G proteins. Shiraishi et al. [25] recently reported the inhibitory effects of alphaxalone on M1R and M3R expressed in *Xenopus* oocytes. Dexmedetomidine has little effect on the M1R function expressing in

Table 3 The effects of Intravenous anesthetics and analgesics on Gq coupled receptor function

	Dex.	Ketamine	Propofol	Pent.	Alph.	Tramadol	ODT
M_1	→	↓	↓		↓	↓	↓
M_3	↓	↓			↓	↓	→
$5HT_{2A}$		→	→	→			
$5HT_{2C}$	→					↓	↓
mGluR1							
mGluR5							
Substance P	→	↓	→	↓		→	↓
Orexin 1	→	↓		↓			

Dex. dexmedetomidine, *Pent.* pentobarbiturate, *Alph.* alfaxalone, *ODT O*-desmethyl tramadol

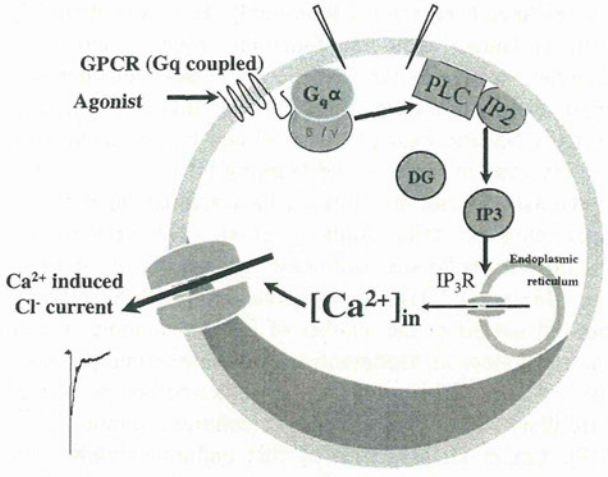

Fig. 2 Intracellular signaling of Gq coupled receptor expressing in *Xenopus* oocytes. Stimulation of G_q coupled receptors results in activation of Ca^{2+}-activated Cl^- currents in *Xenopus* oocytes; stimulation of G_q coupled receptors leads to G protein-dependent activation of phospholipase C, resulting in the formation of IP_3 and diacylglycerol. The IP_3 causes the release of Ca^{2+} from the endoplasmic reticulum, which in turn triggers the opening of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes

Xenopus oocytes expressing M1Rs. In contrast, dexmedetomidine inhibited the ACh-induced currents in *Xenopus* oocytes expressing M3Rs [26].

Local anesthetics also inhibit MRs. Clinically relevant concentrations of lidocaine inhibit M1R signaling [27–29]. Hollmann et al. [27–30] suggested that the major site of action is an extracellular domain of the muscarinic receptor; the N-terminus and third extracellular loop of the M1R molecule were identified as necessary for extracellular inhibition by charged LA, and the intracellular effect of LA most likely takes place at the $G_{\alpha q}$-subunit.

There have been several reports with evidence that showed the effects of analgesics. The effects of tramadol on MRs have been well studied. Information on the effects of tramadol on MRs is scarce. In a rat brain binding experiment, Frink et al. [31] showed that tramadol and its metabolite, *O*-desmethyl tramadol (ODT), have no affinity for M1R. We investigated the effects of tramadol on M1R in two different systems, a *Xenopus* oocyte expression system and on cultured bovine adrenal medullary cells. Tramadol competitively inhibited acetylcholine (ACh)-induced currents in *Xenopus* oocytes expressing the M1R [32]. In cultured bovine adrenal medullary cells, tramadol suppressed muscarine-induced cyclic GMP accumulation and inhibited the specific binding of [^{3}H]-quinuclidinyl benzilate (QNB) [32]. These findings suggest that tramadol inhibits MR function via QNB-binding sites. We also investigated the effects of tramadol on M3R using the *Xenopus* oocytes expression system [33]. Tramadol inhibited ACh-induced currents in oocytes expressing the M3R and the specific binding of [^{3}H]-QNB, suggesting that tramadol inhibits M3R function via QNB-binding sites. This may explain the modulation of neuronal function and the anticholinergic effects of tramadol in clinical situations. To confirm the anticholinergic action of tramadol, we investigated the effects of tramadol on the pH of gastric juices during anesthesia in order to determine whether tramadol inhibits the secretion of gastric juices from gastric glands [34]. After anesthesia was induced, the gastric pH was measured using pH test paper; then tramadol (100 mg), famotidine (20 mg), or saline was injected into the deltoid muscle. The gastric pH was increased by the same amount in both the tramadol and famotidine groups at 3 h after drug administration, suggesting that tramadol inhibits the secretion of gastric acid. The effects of the metabolite ODT on M1R and M3R functions in the *Xenopus* oocytes expression system have been reported [35]; the inhibitory effects of ODT on muscarinic receptors are different from those of tramadol. ODT inhibits M1R function but has little effect on M3R function [35].

As mentioned above, there is much evidence that MRs are the targets of anesthetics and analgesics. By contrast, a recent report pointed out that MRs do not seem to mediate the immobilization caused by inhaled anesthetics [36]. Previous studies have focused on G_q-coupled receptors (M1R and M3R), although, there has been little information on the other MRs, such as M2R. More studies are necessary to reveal the roles of individual MRs in the mechanisms of anesthetics and analgesics.