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Neuropharmacological efficacy of the traditional Japanese Kampo medicine yokukansan and its active ingredients

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ABSTRACT

Dementia is a progressive neurodegenerative disorder with cognitive dysfunction, and is often complicated by behavioral and psychological symptoms of dementia (BPSD) including excitement, aggression, and hallucinations. Typical and atypical antipsychotics are used for the treatment of BPSD, but induce adverse events. The traditional Japanese Kampo medicine yokukansan (YKS), which had been originated from the traditional Chinese medicine *Yi-Gan-San*, has been reported to improve BPSD without severe adverse effects. In the preclinical basic studies, there are over 70 research articles indicating the neuropharmacological efficacies of YKS. In this review, we first describe the neuropharmacological actions of YKS and its bioactive ingredients. Multiple potential actions for YKS were identified, which include effects on serotonergic, glutamatergic, cholinergic, dopaminergic, adrenergic, and GABAergic neurotransmissions as well as neuroprotection, anti-stress effect, promotion of neuroplasticity, and anti-inflammatory effect. Geissoschizine methyl ether (GM) in Uncaria hook and 18β-glycyrrhetinic acid (GA) in Glycyrrhiza were responsible for several pharmacological actions of YKS. Subsequently, we describe the pharmacokinetics of GM and GA in rats. These ingredients were absorbed into the blood, crossed the blood–brain barrier, and reached the brain, in rats orally administered YKS. Moreover, autoradiography showed that [^{3}H]GM predominantly distributed in the frontal cortex and [^{3}H]GA in the hippocampus. Thus, YKS is a versatile herbal remedy with a variety of neuropharmacological effects, and may operate as a multicomponent drug including various active ingredients.

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Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; ADL, activities of daily living; BBB, blood–brain barrier; BPSD, behavioral and psychological symptoms of dementia; BrdU, bromodeoxyuridine; C_{max}, maximum concentration; CHOP, C/EBP homologous protein; CYP, cytochrome P450; DA, dopamine; DLB, dementia with Lewy bodies; DOI, 2,5-dimethoxy-4-iodoamphetamine; ER, endoplasmic reticulum; Erk, extracellular signal-regulated kinase; FDA, Food and Drug Administration; GA, 18β-glycyrrhetinic acid; GABA, gamma-aminobutyric acid; GADD153, growth arrest and DNA damage-inducible gene 153; GLAST, glutamate–aspartate transporter; GLT-1, glutamate transporter-1; GM, geissoschizine methyl ether; GR, glucocorticoid receptor; GRP78/Bip, glucose-regulated protein/ binding immunoglobulin protein; GSH, glutathione; 5-HT, serotonin; MPP^+, 1-methyl-4-phenylpyridine; MPTP, 1-methyl-4-phenyl1,2,3,6-tetrahydropyridene; NMDA, N-methyl-D-aspartate; 8-OH-DPAT, (±)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide; PDD-NOS, pervasive developmental disorder not otherwise specified; PFC, prefrontal cortex; PI3K, phosphatidyl-inositol 3-kinase; PKC, protein kinase C; t_{max}, the time of maximum drug concentration; $t_{1/2}$, half-life; YKS, yokukansan.

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1. Introduction

Dementia is the most frequent age-related neurocognitive disorder. The health care burden and socioeconomic costs of dementia continue to rise with aging population and increased longevity. Behavioral and psychological symptoms of dementia (BPSD), such as excitement, aggression, hallucinations, insomnia, anxiety, wandering, and depression, are observed in 20%–80% patients with dementia (Lawlor, 2004; Cerejeira et al., 2012). In addition, symptom severity is positively associated with care burden (Nagaratnam et al., 1998; Tanji et al., 2005). Typical and atypical antipsychotics have been used for the treatment of BPSD. However, these drugs induce extrapyramidal symptoms and other adverse events, and as a consequence, they decrease the quality of life and increase the difficulty of maintaining activities of daily living (ADL). In 2005, the United States Food and Drug Administration (FDA) warned that patients with dementia-related psychosis treated with atypical antipsychotic drugs are at an increased risk of premature death (FDA, 2005). In addition, conventional antipsychotics are also reported to have the same risk as the atypical antipsychotics (Wang et al., 2005). In 2008, the FDA further warned that both conventional and atypical antipsychotics are associated with an increased risk of mortality in elderly patients treated for dementia-related psychosis (FDA, 2008). Since then, new remedies without adverse effects have been sought.

In such situation, Iwasaki et al. (2005) reported that yokukansan (YKS), a traditional Japanese Kampo medicine, which had been originated from the traditional Chinese medicine *Yi-Gan-San*, improved such excitatory BPSD as hallucinations, agitation, and aggressiveness in 52 patients with Alzheimer's disease (AD), dementia with Lewy bodies (DLB), and other forms of senile dementia, identifying YKS as a new potential therapeutic agent for BPSD. Subsequent clinical trials verified the efficacy of YKS in patients with dementia. For instance, Mizukami et al. (2009) evaluated the efficacy and safety of YKS in 106 patients diagnosed with AD or DLB in a randomized cross-over study and found that YKS was an effective and well-tolerated treatment for patients with BPSD without serious adverse reactions. Monji et al. (2009) demonstrated that YKS was beneficial for treatment of BPSD in 15 elderly patients with AD and suggested that it could also reduce the dose of antipsychotics required for BPSD treatment. Similarly, Okahara et al. (2010) reported high efficacy and safety of YKS in a non-blinded, randomized, parallel-group comparison study of 63 patients with AD. Furthermore, Matsuda et al. (2013) performed a meta-analysis of four randomized controlled trials of YKS for BPSD including 236 patients with dementia (Iwasaki et al., 2005; Mizukami et al., 2009; Monji et al., 2009; Okahara et al., 2010) and concluded that YKS was well tolerated with beneficial effects on neuropsychiatric inventory and ADL scores. In addition to patients with AD (Okahara et al., 2010; Hayashi et al., 2010) and DLB (Iwasaki et al., 2012), YKS has been reported to be effective for BPSD such as agitation and disinhibition in patients with vascular dementia without adverse effects (Nagata et al., 2012). These findings suggest that YKS may improve BPSD regardless of dementia type. YKS has also been shown effective for the symptomatic treatment of other neuropsychiatric disorders, like borderline personality disorder (Miyaoka et al., 2008a), neuroleptic-induced tardive dyskinesia (Miyaoka et al., 2008b), treatment-resistant schizophrenia (Miyaoka et al., 2009), pervasive developmental disorder not otherwise specified (PDD-NOS), Asperger's disorder (Miyaoka et al., 2012), postoperative delirium (Saito et al., 2010), preoperative anxiety (Arai et al., 2014), neuropathic pain (Nakamura et al., 2009), and urticaria/itching (Kato et al., 2010).

In parallel with such clinical investigations, preclinical basic studies have progressed rapidly to clarify the bioactive ingredients and mechanisms of YKS. Kampo medicine is the extract of multiple crude drugs containing a large number of ingredients. Although this molecular complexity is a major impediment to research the therapeutic mechanisms of Kampo medicine, it is an advantage for various pharmacological effects. YKS is composed of seven dried crude drugs, and three-dimensional high-performance liquid chromatographic analysis has identified a plethora of chemical ingredients (Mizukami et al., 2009; Nagata et al., 2012; Tsuji et al., 2014). To date, over 70 basic research articles have been published on the pharmacological efficacy and mechanisms of YKS, its constituent herbs, and ingredients, and the pharmacokinetics, metabolism, and brain distribution of active ingredients. In this review, we describe the neuropharmacology of YKS, focusing on these basic studies.

2. Yokukansan (YKS)

YKS originally appeared as *Yi-Gan San* in the "*Bâo yïng cuö yào*", the Synopsis for Protecting Infant written by Xue Kai during the Ming dynasty (1555) in China as a remedy for restlessness and agitation in children. It was introduced to Japan in the late 17th century, and a formula for use in adults was developed in the late 18th century. At present, YKS is in widespread use as a traditional herbal medicine, which has approved by the Japanese Ministry of Health, Labor and Welfare as a remedy for neurosis, insomnia, and irritability and night crying in children. It is composed of seven dried medicinal herbs, Atractylodes lancea rhizome (4.0 g, rhizome of *Atractylodes lancea* De Candolle), Poria sclerotium (4.0 g, sclerotium of *Poria cocos* Wolf), Cnidium rhizome (3.0 g, rhizome of *Cnidium officinale* Makino), Uncaria hook (3.0 g, thorn of *Uncaria rhynchophylla* Miquel), Japanese Angelica root (3.0 g, root of *Angelica acutiloba* Kitagawa), Bupleurum root (2.0 g, root of *Bupleurum falcatum* Linné), and Glycyrrhiza (1.5 g, root and stolon of *Glycyrrhiza uralensis* Fisher). Each plant material is identified by its external morphology and authenticated by marker compounds of plant specimens according to the methods of the Japanese Pharmacopoeia and our company's standard. To produce the dried YKS extract powder, the mixture of seven component herbs is extracted with purified hot water at 95 °C for 1 h. The extract solution is separated from the insoluble waste and spray-dry to produce the dried extract powder. The quality is standardized based on Good Manufacturing Practice defined by the Ministry of Health, Labour and Welfare of Japan. As shown in Fig. 1, we have identified at least 25 ingredients in the methanol fraction of YKS extract by three-dimensional high-performance liquid chromatographic analysis with ultraviolet detection (Mizukami et al., 2009; Nagata et al., 2012; Tsuji et al., 2014).

3. Neuropharmacological actions

Several reports have shown the neuropharmacological actions of YKS, which include the effects on serotonergic, glutamatergic, cholinergic, dopaminergic, adrenergic, and gamma-aminobutyric acid (GABA)ergic neural systems, as well as neuroprotective effect, anti-stress effect, promotion of neuroplasticity, and anti-inflammatory effect. These are thought to be associated with the therapeutic efficacies of YKS on BPSD (Fig. 2).

3.1. Serotonergic neurotransmission

3.1.1. Serotonin 1A (5-HT_{1A}) receptor

As described, YKS ameliorated positive BPSD-like aggressiveness in clinical trials (Iwasaki et al., 2012; Matsuda et al., 2013). In animals, aggressive behaviors are related to dysfunction of the cerebral serotonergic system (Nichols, 2004). In fact, we demonstrated a significant increase in aggressive behavior and decrease in social behavior in cerebral 5-HT-deficient rats treated with the 5-HT neurotoxin ρ-chloroamphetamine (Kanno et al., 2009). YKS ameliorated this neurotoxin-induced aggressive behavior and impaired social behavior as effectively as the 5-HT_{1A} receptor partial agonist buspirone and the 5-HT_{2A} receptor antagonist ketanserin. From this result, we speculated tentatively that the ameliorative effects of YKS may be due to agonism of the 5-HT_{1A} receptor or antagonism of the 5-HT_{2A} receptor.

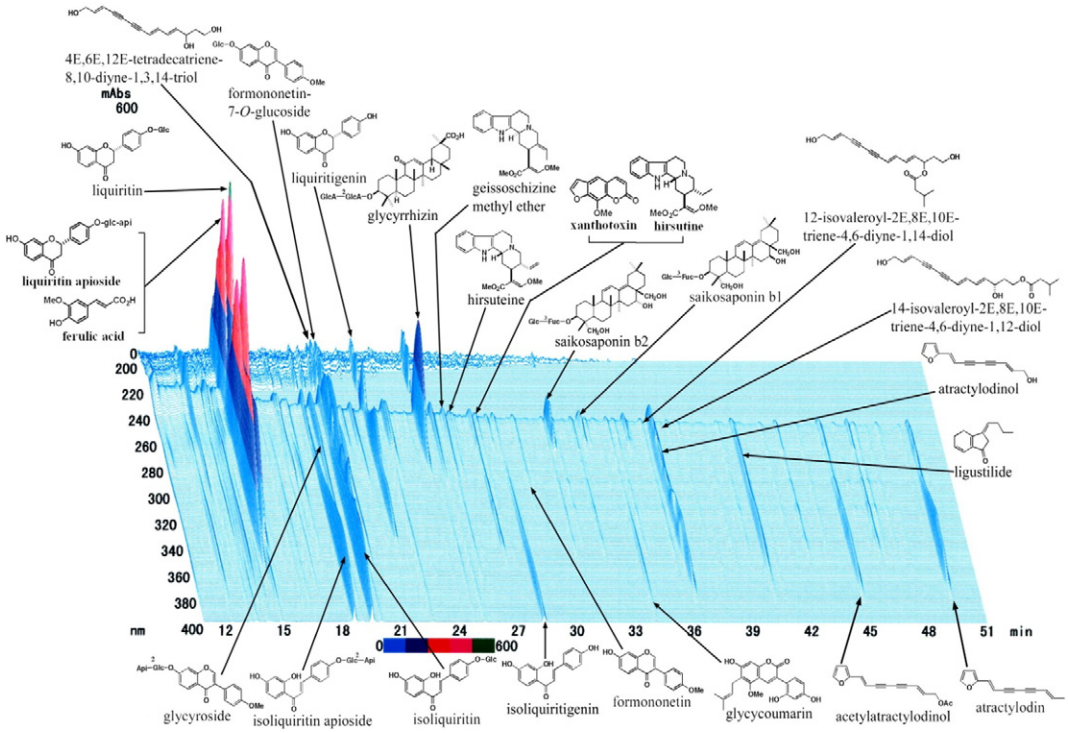

Fig. 1. Three-dimensional chromatogram of YKS extract. The dried extract (1 g) of YKS was dissolved in 20 ml of methanol under ultrasonication for 30 min and centrifuged at 3000 rpm for 5 min. The supernatant was filtrated through a 0.45 μm membrane filter. A 30 μl sample of the filtrate was injected into a high-performance liquid chromatography system with ultraviolet detection.

To distinguish between these possibilities, we conducted a competitive binding assay for 5-HT receptors in membranes of Chinese hamster ovary cell stably expressing human recombinant 5-HT_{1A} or 5-HT_{2A} receptors (Terawaki et al., 2010). YKS competitively inhibited [^{3}H](±)-8-hydroxy-2-(dipropylamino)tetralin hydrobromide (8-OH-DPAT) binding to the 5-HT_{1A} receptor but not [^{3}H]ketanseri binding to 5-HT_{2A} receptor. The [^{35}S]GTPγS binding assay showed that YKS had a partial agonistic effect on the 5-HT_{1A} receptor, as the maximal [^{35}S]GTPγS binding rate in the presence of YKS was approximately 50% of that of a full agonist 5-HT. Similar binding characteristics were found only for Uncaria hook among the seven constituent herbs of YKS. Furthermore, the binding characteristics of YKS to the 5-HT_{1A} receptor were markedly attenuated by eliminating Uncaria hook from YKS but were almost unchanged when other components were individually eliminated. A subsequent *in vitro* binding assay to identify the active ingredients from Uncaria hook showed that only geissoschizine methyl ether (GM) among seven alkaloids strongly bound to the 5-HT_{1A} receptor and acted as a partial agonist with efficacy similar to YKS and Uncaria hook (Nishi et al., 2012).

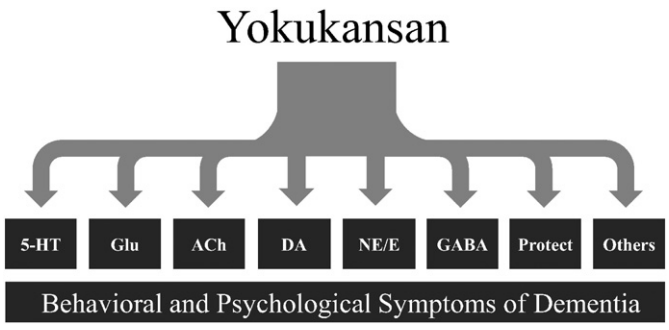

Fig. 2. Neuropharmacological actions of YKS. 5-HT, serotonergic neurotransmission; Glu, glutamatergic neurotransmission; ACh, cholinergic neurotransmission; DA, dopaminergic neurotransmission; NE/E, adrenergic neurotransmission; GABA, GABAergic neurotransmission, Protect: Neuroprotection; "Others" include anti-stress, neuroplasticity, neurogenesis, and anti-inflammation.

We next aimed to verify these *in vitro* results by examining the efficacy of Uncaria hook for ameliorating aggression and reduced social behaviors in socially isolated mice (Nishi et al., 2012). The mitigating effect of YKS (1.0 g/kg) was completely abolished by removal of Uncaria hook, and oral administration of 150 mg/kg Uncaria hook, the equivalent amount in 1.0 g/kg YKS, also significantly reduced aggression and promoted social behaviors as effectively as YKS. Further, oral administration of 150 μg/kg GM, the equivalent amount in 1.0 g/kg YKS, also ameliorated aggressiveness and reduced sociality as effectively as YKS and Uncaria hook. These effects of YKS, Uncaria hook, and GM were counteracted by co-administration of the 5-HT_{1A} receptor antagonist WAY-100635. These results suggest that GM in Uncaria hook is a potent 5-HT_{1A} receptor agonist and a candidate bioactive agent mediating the effects of YKS on aggression and reduced sociality induced by social isolation. Other *in vivo* studies also reported that WAY-100635 counteracted the ameliorative effects of YKS against aggressive behavior, reduced social behavior, and anxiety observed in various animal models (Kanno et al., 2009; Nishi et al., 2012; Yamaguchi et al., 2012).

Interestingly, Ueki et al. (2015a) demonstrated that YKS increased the density of 5-HT_{1A} receptor in the prefrontal cortex (PFC) of socially isolated mice and also enhanced the behavioral response (i.e., decreased rearing behavior) induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT, suggesting that YKS increases 5-HT_{1A} receptor expression and functional activity. Considering that GM behaves as a partial agonist for 5-HT_{1A} receptors, YKS is suggested to have structure in its formula to enhance the effects of GM by upregulating 5-HT_{1A} receptor expression.

3.1.2. Serotonin 2A (5-HT_{2A}) receptor and other subtype receptors

Egashira et al. (2008) reported that oral administration of YKS to normal mice ameliorated the 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head-twitch response (a hallucination-like behavior in mice) by downregulating 5-HT_{2A}

receptor expression in the PFC as measured by Western blotting. More recently, we demonstrated that YKS reduced the enhanced DOI-induced head-twitch response in isolation-stressed mice and downregulated the increased 5-HT_{2A} receptor density in the PFC of these stressed mice (Ueki et al., 2015b); in this study, the downregulation of 5-HT_{2A} receptors is suggested to be induced by a synergistic effect of Bupleurum root, Uncaria hook, Japanese Angelica root, and Glycyrrhiza among the seven constituent components of YKS. Thus, the ameliorative effect of YKS on hallucination-like behaviors may result from 5-HT_{2A} receptor downregulation by multiple YKS components, although the exact mechanisms are currently unclear. Carrasco et al. (2007) and Wieland et al. (1993) demonstrated that activation of 5-HT_{1A} receptors desensitizes 5-HT_{2A} receptors, suggesting that YKS may induce 5-HT_{2A} receptor downregulation through partial agonistic action on 5-HT_{1A} receptors.

Ueda et al. (2011) reported in a single-cell-based Ca^{2+} imaging assay that GM, which is a candidate for pharmacological effect of YKS, has third-generation antipsychotic-like actions at the 5-HT and dopamine (DA) receptors, i.e., GM behaves as a partial agonist at the 5-HT_{1A} receptor, an antagonist at the 5-HT_{2A}, 5-$HT_{2C,}$ and 5-HT_7 receptors, and a partial agonist/antagonist at the D_{2L} receptor, like aripiprazole. Ueki et al. (2013) also reported that GM has an antagonistic effect on the 5-HT_7 receptor as determined by measuring intracellular cAMP levels in HEK293 cells stably expressing human recombinant 5-HT_7 receptor. With regard to the 5-HT_{2A} receptor, although we could not detect the 5-HT_{2A} antagonist effect in experiments using YKS extract, GM had measurable 5-HT_{2A} antagonist activity. This may be due to the differences in crude extract YKS and single-ingredient GM. As described in detail below, the evidence that GM is detected in the blood and brain of rats orally administered YKS (Imamura et al., 2011) suggests that YKS has multiple actions on 5-HT receptors through GM.

In addition to GM, Uncaria hook contains other indole alkaloids such as hirsuteine and hirsutine, as well as oxindole alkaloids like rhynchophylline, isorhynchophylline, corynoxeine, and isocorynoxeine (Fig. 3). These oxindole alkaloids have a carbonyl group at the C2

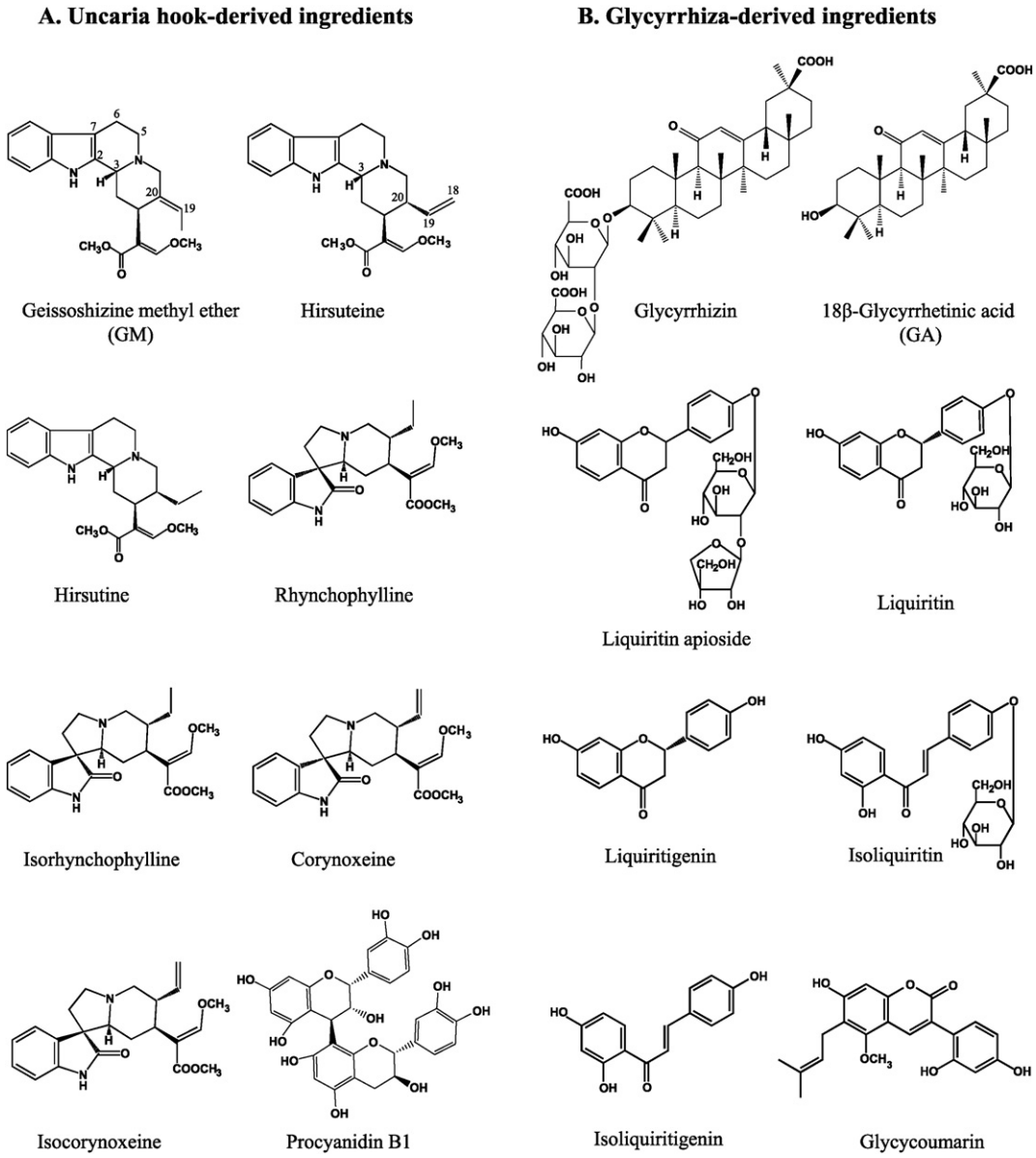

Fig. 3. Chemical structures of (**A**) Uncaria hook-derived indole alkaloids (geissoschizine methyl ether (GM), hirsuteine, and hirsutine), oxindole alkaloids (rhynchophylline, isorhynchophylline, corynoxeine, isocorynoxeine), and flavonoid (procyanidin B1), and (**B**) Glycyrrhiza-derived triterpenoids (glycyrrhizin and 18β-glycyrrhetinic acid (GA)) and flavonoids (liquiritin apioside, liquiritin, liquiritigenin, isoliquiritin, isoliquiritigenin, and glycycoumarin).

position of the five carbon ring of the indole alkaloid structure and showed no binding activity to 5-HT_{1A} and 5-HT_7 receptors. Alternatively, GM showed strong binding activity for these receptors, while hirsuteine, which has very similar structure to GM, did not bind to them. As shown in Fig. 3, the conformations at C3 and the side chain at C20 (GM has a double bond at C19–20 and hirsuteine at C18–19) differ and the resulting steric change may relate to 5-HT_{1A} or 5-HT_7 receptor binding activity.

3.1.3. Serotonin (5-HT) release

Mizoguchi et al. (2010) reported that YKS reversed the decreased 5-HT release in the PFC of aged rats, which might be associated with the anxiolytic effects of YKS. However, the active ingredients have not yet identified.

3.2. Glutamatergic neurotransmission

3.2.1. Glutamate release

Zinc deficiency has been shown to increase glutamate concentration in the brain tissue of mice and extracellular glutamate concentration in the hippocampus of rats (Takeda et al., 2008a; Tamano et al., 2010). Moreover, social isolation in zinc-deficient mice induced aggressive behavior. YKS ameliorated this aggressive behavior and normalized the concomitant increased glutamate concentrations (Takeda et al., 2008a; Tamano et al., 2010; Takeda et al., 2012). This normalization of glutamate concentrations is due to inhibition of excess glutamate release via reduced exocytosis in the hippocampus (Takeda et al., 2008b). More recently, Tamano et al. (2016) suggested that GM and 18β-glycyrrhetinic acid (GA), a major metabolite of glycyrrhizin contained in Glycyrrhiza, ameliorated excess glutamate release from mossy fiber boutons by suppressing the increase in intracellular Ca^{2+} signaling.

3.2.2. Glutamate transport

We demonstrated that thiamine deficiency induced memory disturbances and BPSD-like symptoms including anxiety, aggressive behavior, and reduced social behavior in rats (Ikarashi et al., 2009; Iizuka et al., 2010). Electron microscopy of the brain showed more severe degeneration of astrocytes than neurons in several brain regions, including brain stem, hippocampus, and cerebral cortex. A microdialysis study revealed elevated extracellular glutamate in the thiamine-deficient brain. YKS prevented the development of memory disturbances and BPSD-like behaviors, astrocyte degeneration, and increased brain glutamate level resulting from thiamine deficiency. Considering that astrocytes have a role in removal of excessive extracellular glutamate, these observations suggest that thiamine deficiency enhances extracellular glutamate by reducing astroglial-mediated uptake via glutamate transporters, and YKS prevents these dysfunctions. Indeed, YKS ameliorated the thiamine deficiency-induced decrease in both glutamate uptake into in cultured rat astrocytes and the concomitant reduction in expression of glutamate–aspartate transporter (GLAST) mRNA and protein (Kawakami et al., 2009). Moreover, the ameliorative effect of YKS against reduced glutamate transport was completely abolished by the glutamate transporter inhibitor DL-threo-β-hydroxy-aspartic acid. Taken together, these results strongly suggest that YKS protects astrocytes from damage induced by thiamine deficiency, thereby preserving glutamate transporter function.

Among the seven constituent herbs of YKS, a significant promotion of glutamate transport was found for Glycyrrhiza. Furthermore, we found that glycyrrhizin and GA among eight ingredients (Fig. 3) of Glycyrrhiza ameliorated the thiamine deficiency-induced decrease in cultured astroglial glutamate uptake in a concentration-dependent manner (Kawakami et al., 2009, 2010). Glutamate transporter expression and glutamate transport activity are decreased by protein kinase C (PKC)-activating phorbol esters (Conradt & Stoffel, 1997; Gonzalez et al., 1999), and these ingredients inhibited PKC activity. In addition, both reductions of glutamate uptake and glutamate transporter protein expression in astrocytes under thiamine deficiency were reversed by treatment with the PKC inhibitor H-7 (Hazell et al., 2003). These lines of evidence suggest that glycyrrhizin and GA are likely responsible for the ameliorative effects of YKS on dysfunction of glutamate transport in astrocytes by inhibiting PKC.

Glutamate-related mechanisms of YKS have been reported in other animal models. In rats exhibiting haloperidol-induced tardive dyskinesia, YKS ameliorated the associated increase in striatal extracellular glutamate by increasing the expression of glutamate transporter mRNA (Sekiguchi et al., 2012). In the rat chronic constriction injury model of neuropathic pain, YKS inhibited the brush- and acetone-induced increase in cerebrospinal fluid glutamate concentration, and these anti-allodynia actions were counteracted by intrathecal injection of the nonspecific glutamate transporter-1 (GLT-1) and GLAST inhibitor DL-threo-β-hydroxy-aspartic acid and by the selective GLT-1 inhibitor dihydrokainate (Suzuki et al., 2012). Facilitating actions of GA on glutamate uptake may be involved in these effects of YKS.

Control of glutamate, the predominant fast excitatory neurotransmitter in the brain, is also critical for inhibition of neurotoxicity (excitotoxicity), and YKS has neuroprotective effects. These are described in the section of neuroprotection below.

3.3. Cholinergic neurotransmission

In vitro biochemical assays showed that YKS did not affect acetylcholinesterase and choline acetyltransferase activities or bind to cholinoreceptors and choline transporter (Sekiguchi et al., 2011). However, hippocampal acetylcholine release was increased by YKS treatment in rats subjected to cerebrovascular ischemia (Nogami et al., 2013) and amyloid β (Aβ) protein intracerebroventricular injection plus ischemia/reperfusion injury (Uchida et al., 2013). These authors suggested that the acetylcholine-releasing effects of YKS are related to an increase in dynamin 1, which is known to play a critical role in synaptic vesicle recycling, and are associated with amelioration of learning and memory dysfunction. There are several studies that have demonstrated the usefulness of other Kampo medicines (chotosan and tokishakuyakusan) in the treatment of cognitive disorders. As these medicines contain constituent herbs that are similar to those (Uncaria hook in chotosan, and Atractylodes lancea rhizome, Poria sclerotium, Cnidium rhizome, Japanese Angelica root in tokishakuyakusan) in YKS, they (Uchida et al., 2013) are inferring that the overlapped constituents may be active components. Unfortunately, the active ingredients responsible for the expression of dynamin 1 remain unknown.

3.4. Dopaminergic neurotransmission

Although GM behaves as a partial agonist/antagonist at the D_{2L} receptor (Ueda et al. 2011), an *in vitro* competitive binding assay showed that YKS itself did not affect the binding of DA receptor subtype-specific radioligands (Sekiguchi et al., 2012). Extrapyramidal symptoms such as inhibition of motor activity and catalepsy, which are induced by a DA deficit or DA receptor blockade, were not observed in various animal models treated with YKS (Sekiguchi et al., 2009; Kanno et al., 2009; Nishi et al., 2012). Mizoguchi et al. (2010) reported that YKS improved the age-related decrease in dopaminergic transmission (as evidenced by increased extracellular concentrations) in the PFC, which might be associated with the ameliorative effects of YKS on age-related cognitive impairments (Mizoguchi et al., 2011). More recently, Ishida et al. (2016) reported that YKS facilitated DA supplementation by L-DOPA via catechol-*O*-methyltransferase inhibition in rats with unilateral 6-hydroxydopamine lesions in the nigrostriatal dopaminergic pathway. They suggested that the Uncaria hook alkaloids GM and corynoxeine may contribute these effects of YKS.

3.5. Adrenergic neurotransmission

Action of YKS on adrenergic neurotransmission has been studied only in the context of morphine dependence in mice (Nakagawa et al., 2012). YKS significantly attenuated morphine tolerance and naloxone-precipitated morphine withdrawal signs (jumps and body weight loss) without affecting the analgesic effect of morphine. The inhibitory effect of YKS on morphine withdrawal jumps was blocked by a single pretreatment with the α_2-adrenoceptor antagonist yohimbine but not by the α_1-adrenoceptor antagonist prazosin. A similar inhibitory effect on withdrawal jumps was observed by repeated administration of yohimbine. Plasma membrane α_{2A}-adrenoceptor expression in the pons–medulla was reduced during morphine withdrawal, and this reduction was prevented by repeated administration of YKS or yohimbine. An *in vitro* receptor binding assay demonstrated that YKS and two of its constituent herbs, Uncaria hook and Glycyrrhiza, revealed an antagonistic effect for α2A adrenoceptor and that certain chemical ingredients, including GM, glycyrrhizin, and GA shared this activity. Furthermore, administration of Uncaria hook, Glycyrrhiza, or their respective ingredients (GM or glycyrrhizin) significantly inhibited morphine withdrawal signs. These results suggest that GM and glycyrrhizin in YKS inhibit morphine tolerance and physical dependence by blocking α_{2A}-adrenoceptors, thereby preventing decreased receptor expression in the brainstem.

3.6. GABAergic neurotransmission

YKS has been reported to reverse the shortening of pentobarbital-induced sleep time in socially isolated mice (Egashira et al., 2011). This effect is thought to involve the $GABA_A$-benzodiazepine receptor complex but not 5-HT_{1A} receptors because reversal was blocked by the $GABA_A$ receptor antagonist bicuculline and the selective benzodiazepine receptor antagonist flumazenil but not by the selective 5-HT_{1A} receptor antagonist WAY 100635. Kamei et al. (2009) suggested that the anxiolytic-like effect of YKS may be mediated by $GABA_A$ receptor stimulation and that the effective ingredients are contained in the water-soluble fraction of YKS. Liao et al. (1995) reported that the water extract of Japanese Angelica root, a constituent herb of YKS, bound to $GABA_A$ receptors *in vitro*. However, we have found that YKS includes water-soluble botanic GABA (Nishi et al., 2012). When we completely washed out the GABA contained in YKS using distilled water and then obtained the methanol extract, an *in vitro* binding assay showed no binding to either the GABA or benzodiazepine binding site. These results suggest that the binding of YKS against $GABA_A$/benzodiazepine receptor is due to botanic GABA in YKS because the active ingredients were not included in the methanol extract. However, orally administrated GABA does not pass efficiently across the blood–brain barrier (BBB) (Cooper et al., 1991). Therefore, we suggest that YKS does not directly act on $GABA_A$/benzodiazepine receptors in the brain. Rather, the effects of YKS on the receptors may be indirect, for example, allopregnanolone, a potent positive allosteric modulator of GABA actions at the $GABA_A$ receptor, may be involved in the effects of YKS (Matsumoto et al., 1999).

3.7. Neuroprotection

3.7.1. Glutamate neurotoxicity

Glutamate is the major fast excitatory neurotransmitter in the central nervous system, and synaptic plasticity at glutamatergic synapses is essential for certain forms of learning and memory. However, high extracellular glutamate concentrations or prolonged elevation induces excitotoxic neuronal death (Choi, 1988; Cheung et al., 1998). As already described, YKS mitigates the disruption of astroglial glutamate transport, thereby preventing excitotoxicity. In addition, YKS has been demonstrated to have a direct protective effect on neurons (Kawakami et al., 2011a); YKS (100 μg/mL) protected primary cultured rat cortical neurons against 100 μM glutamate-induced neuronal death. Among the seven constituent herbs of YKS, highest potency of protection was found for Uncaria hook and Glycyrrhiza, suggesting that these herbs contain neuroprotective ingredients. Fig. 3 shows the ingredients examined for neuroprotective efficacy. Four ingredients in Uncaria hook (10 μM GM, hirsuteine, hirsutine, and rhynchophylline) and four ingredients in Glycyrrhiza (10 μM glycycoumarin, isoliquiritigenin, liquiritin, and glycyrrhizin) showed neuroprotective efficacy, suggesting that individual ingredients additively and/or synergistically contribute to the neuroprotective effect of YKS. In N-methyl-D-aspaetate (NMDA) receptor binding and receptor-linked Ca^{2+} influx assays, only isoliquiritigenin bound to and antagonized NMDA receptors and inhibited glutamate-induced Ca^{2+} influx. However, the neuroprotective effect of isoliquiritigenin might not be mediated by NMDA receptor antagonism because the concentration needed for substantial NMDA binding (>100 μM) and inhibition of Ca^{2+}-influx (300 μM) were higher than that for its neuroprotective effect (10 μM). These findings suggested that it was difficult to explain the neuroprotective effect of YKS by NMDA receptor antagonism of isoliquiritigenin and that mechanisms other than NMDA receptor antagonism must account for the neuroprotective efficacy of YKS.

3.7.2. Antioxidant effect of yokukansan (YKS) mediated by the cysteine/glutamate antiporter system Xc^-

Regarding glutamate neurotoxicity, two mechanisms have been proposed; glutamate receptor-mediated neurotoxicity and cystine/glutamate antiporter system Xc^- inhibition-mediated neurotoxicity (Choi, 1988; Murphy et al., 1990; Schubert et al., 1992; Froissard & Duval, 1994; Pereira & Oliveira, 2000; Penugonda et al., 2005; Edwards et al., 2007; Lo et al., 2008). The system Xc^- imports the amino acid cystine, an oxidized form of cysteine, into cells with 1:1 counter-transport of glutamate. It consists of two protein components, the 4F2 heavy chain necessary for membrane localization of the heterodimer and the xCT protein responsible for transporter activity. Cysteine is the rate-limiting substrate for synthesis of the antioxidant glutathione (GSH), along with cystine (Lo et al., 2008; Conrad & Sato, 2012; Lewerenz et al., 2012, 2013). PC12 cells express the system Xc^- but not a normal NMDA receptor subunit profile (Pereira & Oliveira, 2000; Penugonda et al., 2005; Edwards et al., 2007; Lo et al., 2008; Vazhappilly et al., 2010). Furthermore, glutamate-induced cell death in PC12 cells was not blocked by 100 μM MK-801, which is sufficient to block glutamate-induced neuronal death (Lysko et al., 1989). Conversely, 100 μM NMDA, which is sufficient to induce neuronal death (Zhu et al., 2003), did not induce cell death in PC12 cells. Previous reports have shown that PC12 cells do not express functional NMDA receptors (Kawakami et al., 2011b). These findings suggest that PC12 cell death induced by glutamate is not due to stimulation of NMDA receptors and that the PC12 cell is a valuable model to selectively evaluate the improving effects on glutamate-mediated cytotoxicity through system Xc^- inhibition. We found that YKS had cytoprotective effect against glutamate-mediated cytotoxicity in PC12 cells (Kawakami et al., 2011b; Kanno et al., 2014). When the effects of the seven constituent herbs in YKS on PC12 cell death were examined separately, Uncaria hook was found to have the highest cytoprotective potency, and the Uncaria hook ingredients GM, hirsutine, hirsuteine, and procyanidin B1 all had cytoprotective effects. These ingredients enhanced gene expression of system Xc^- subunits xCT and 4F2 heavy chain and ameliorated the glutamate-induced decrease in the antioxidant GSH levels. The cytoprotective effects of YKS and these four Uncaria hook-derived ingredients were counteracted by co-treatment with the system Xc^- inhibitor (S)-4-carboxyphenylglycine. These results suggest that the enhancement of system Xc^- gene expression by these four ingredients may contribute, at least in part, to the cytoprotective efficacy of YKS by preserving cellular antioxidant capacity (GSH cycling). A similar protective effect of YKS against GSH reduction was observed in the brain of mice treated with polyinosinic–polycytidilic acid (Makinodan

et al., 2009). More recently, GM has been reported to protect glutamate-induced neurotoxicity in cultured cells through suppressing reactive oxygen species generation and upregulating glycolysis (Sun et al., 2016).

3.7.3. Amyloid β (Aβ) neurotoxicity

In the brains of patients with AD, Aβ aggregates and forms insoluble fibrils that are the major component of senile plaques. Recently, Aβ oligomers have been focused as a mechanism of the pathology for AD because of its neurotoxicity both *in vivo* and *in vitro*. YKS has been demonstrated to suppress Aβ oligomer-induced neuronal apoptosis in primary cultured cortical neurons (Tateno et al., 2008; Kanno et al., 2013). Moreover, we found that glycycoumarin in Glycyrrhiza and procyanidin B1 in Uncaria hook contribute to the neuroprotective effect of YKS through the suppression of the apoptosis effector caspase-3 (Kanno et al., 2015).

With regard to Aβ aggregation, Fujiwara et al. (2006) reported that Uncaria hook has a potent anti-aggregation effect on Aβ proteins *in vitro*. Tabuchi et al. (2009) examined the effects of YKS on Aβ accumulation in the brain of amyloid precursor protein-transgenic mice, and showed that YKS did not inhibit histological deposition of Aβ and production of soluble and insoluble forms of Aβ. On the other hand, Fujiwara et al. (2011) showed that YKS and Uncaria hook prevented the accumulation of cerebral Aβ in the same animal model. At present, the *in vivo* effects of YKS against Aβ deposition have been controversial, but both studies demonstrated that YKS prevented memory disturbance and aggressive behavior. These preventative effects of YKS may be mediated by the mechanisms related to neuroprotection and neurotransmission rather than anti-Aβ aggregation effects, and this may be attributed to Uncaria hook.

3.7.4. 1-Methyl-4-phenylpyridine (MPP^+)/ 1-methyl-4-phenyl1,2,3,6-tetrahydropyridene (MPTP) neurotoxicity

Doo et al. (2010) investigated the neuroprotective effects of YKS against MPP^+/MPTP-induced cytotoxicity *in vitro* and *in vivo*. Pretreatment of SH-SY5Y human neuroblastoma cells with YKS protected against MPP^+-induced cell death and significantly decreased caspase-3 activity concomitant with increased expression of phospho-activated Akt (protein kinase B). An inhibitor of phosphatidyl-inositol 3-kinase (PI3K), LY294002, significantly decreased this protective effect of YKS. In mice, YKS treatment also significantly improved motor dysfunction and prevented dopaminergic neuronal loss related to MPTP challenge, a model of Parkinson's disease. These results suggest that YKS can rescue dopaminergic neurons from MPP^+/MPTP toxicity via activation of the PI3K/Akt signaling pathway. Recently, Kubota et al. (2013) demonstrated in PC12 cells that YKS induced nerve growth factor-like phosphorylation and activation of protein kinase and lipid kinase pathways, including extracellular signal-regulated kinase 1/2 and PI3K/Akt pathways known to regulate neuronal protection, proliferation, and differentiation (Vaudry et al., 2002; Chen et al., 2012), as well as neurite outgrowth (Tsuji et al., 2001). β-Eudesmol, a sesquiterpenoid isolated from Atractylodes lancea rhizome, is suggested to be one of candidates for neurotrophic effects (Obara et al., 2002; Kubota et al., 2013).

3.7.5. Endoplasmic reticulum (ER) stress

ER stress is known to activate two pathways; survival and apoptotic pathways. The survival pathway involves the increased transcription of genes encoding ER stress-resident chaperones, including glucose-regulated protein/binding immunoglobulin protein (GRP78/Bip), GRP94, and protein disulfide isomerase, which facilitate protein folding (Kozutsumi et al., 1988; Sidrauski et al., 1998; Tirasophon et al., 1998; Oyadomari et al., 2002), while the apoptotic pathway involves the induction of C/EBP homologous protein (CHOP), also known as growth arrest and DNA damage-inducible gene153 (GADD153) (Oyadomari et al., 2002; Oyadomari & Mori, 2004). YKS prevented the death of SK–N–SH human neuroblastoma cells and neuro-2a mouse neuroblastoma cells resulting from ER stress induced by hypoxic exposure or thapsigargin (Hiratsuka et al., 2010). It was suggested that these protective effects are mediated by both upregulation of GRP78/Bip expression and inhibition of CHOP induction. In addition, YKS and Cnidium rhizome inhibited caspase-4 activation under ER stress. Ferulic acid contained in Cnidium rhizome was suggested to play an important role in this protective effect of YKS.

3.8. Anti-stress effect

Severe and accumulative stress is thought to participate or exacerbate anxiety, depression, and cognitive deficits in humans and animals (Anisman & Zacharko, 1990; Mazure, 1995; Mizoguchi et al., 2000). Stress activates the hypothalamic–pituitary–adrenal axis, resulting in the release of glucocorticoid hormones that enhance adaptive capacity to stress (Sapolsky et al., 1986; Porter et al., 2001). However, excessive glucocorticoids are considered to be cytotoxic to neurons. As described above, repeated administration of YKS attenuated stress-induced neuronal excitation in the rat PFC and amygdala as measured by c-Fos expression (Shoji & Mizoguchi, 2013). Nakatani et al. (2014) also reported that YKS protected corticosterone-induced neurotoxicity in mouse primary cultured hippocampal neurons. More recently, Shimizu et al. (2015a,b) demonstrated that YKS normalized the stress-induced elevation of plasma corticosterone, possibly through a microRNA-dependent mechanism. MicroRNAs are noncoding RNAs that inhibit the translation and/or decrease the stability of their target mRNAs, ultimately decreasing target protein expression (Carthew, 2006; Wang et al., 2007). MicroRNA-18 and/or 124a are candidate negative regulators of glucocorticoid receptor (GR) expression in the brain (Vreugdenhil et al., 2009), and enhanced GR signaling attenuates the hypothalamic–pituitary–adrenal axis activity. In mice, YKS reversed the stress-induced decrease in GR proteins in the paraventricular nucleus of the hypothalamus and oligodendrocytes of the corpus callosum. YKS also decreased microRNA-18 in the paraventricular nucleus and microRNA-124a in callosal oligodendrocytes, although GR mRNA level was not significantly changed in either region (Shimizu et al., 2015a,b). These results suggest that YKS normalizes the stress-induced decrease in GR proteins by downregulating microRNA-18 and 124a expression levels and subsequently disinhibiting GR mRNA translation.

3.9. Neuroplasticity, neurogenesis, proliferation, and differentiation

Extracellular matrix-associated glycoproteins such as chondroitin sulfate proteoglycans regulate the proliferation, migration, and neurite outgrowth of neural stem cells in the brain and act to maintain the structure and function of adult neurons. Tanaka & Mizoguchi (2009) examined the influence of aging and the improving effects of YKS on the expression of aggrecan, a major chondroitin sulfate proteoglycan, on the proliferation and migration of neural stem/progenitor cells identified by bromodeoxyuridine (BrdU) incorporation in the PFC and hippocampus/dentate gyrus of aged rats. In the aged rat PFC and hippocampus, increased aggrecan expression and decreased numbers of BrdU-labeled cells were observed, and these changes were reversed by YKS treatment. These results suggest that aging influences the microenvironment for adult and immature neurons in the brain, which may affect the proliferation and migration of neural stem/progenitor cells, while YKS induces growth-permissive changes in the brain microenvironment. This enhanced neurogenesis was also demonstrated in the dentate gyrus of YKS-treated Gunn rats (Furuya et al., 2013). In addition, YKS promoted neurite outgrowth in PC12 cells through activation of extracellular signal-regulated kinase (Erk)1/2 and PI3K/Akt as described above (Kubota et al., 2013).

YKS also promoted the proliferation of purified mouse cortical oligodendrocyte precursor cells and their differentiation into

oligodendrocytes, and GM was one of active ingredients responsible for this effect (Ueki et al., 2014). This result was supported by Morita et al. (2014), who examined the effect of GM on demyelination in cuprizone-treated mice, and demonstrated that GM significantly increased BrdU-labeled GSTpi$^+$ mature oligodendrocytes and attenuated the decrease in myelin basic protein immunoreactivity in the medial PFC. From these results, they suggested that GM may enhance both proliferation of oligodendrocyte precursor cells and their differentiation into oligodendrocytes.

3.10. Anti-inflammatory effect

Liu et al. (2014) examined the anti-inflammatory and antioxidant properties of YKS in gerbils subjected to cerebral ischemia/reperfusion injury. YKS attenuated the injury-induced inflammatory response (the increased number of Iba1 immunoreactive microglia), DNA oxidative damage (a high level of 8-hydroxy-2′-deoxyguanisine) and subsequent neuronal apoptosis in the hippocampal CA1 area. Furuya et al. (2013) demonstrated that YKS attenuated microglial activation as evaluated by enhanced CD11b immunoreactivity in Iba1-labeled cells, and promoted neurogenesis in the hippocampal dentate gyrus of Gunn rats. In addition, several ingredients in YKS possess potential anti-inflammatory property; rhynchophylline and isorhynchophylline in Uncaria hook (Yuan et al., 2009; Song et al., 2012), GA and liquiritigenin in Glycyrrhiza (Khaksa et al., 1996; Kim et al., 2008), saikosaponin a, c, d, e in Bupleurum root (Lu et al., 2012), β-eudesmol in Atractylodes lancea rhizome (Seo et al., 2011), senkyunolide A, Z-ligustilide, and ferulic acid in Cnidium rhizome (Cheng et al., 2008; Or et al., 2011), pachymic acid in Poria scletotium (Nukaya et al., 1996). Taken together, these findings suggest that the pharmacological activity of YKS may be mediated in part by an anti-inflammatory effect.

4. Pharmacokinetics

4.1. Detection of geissoschizine methyl ether (GM) and 18β-glycyrrhetinic acid (GA) in the plasma and brain, and blood–brain barrier permeabilities

We have described multiple psychotropic effects of YKS and constituent components and their pharmacological mechanisms. While it is possible that many active ingredients remain to be identified, several lines of evidence suggest that GM and GA are major contributors to the pharmacologic efficacy of YKS. Subsequent pharmacokinetics studies demonstrated that GM and GA were detected in the plasma of rats orally administered YKS (0.5–4.0 g/kg). The maximum concentration (C_{max}) of GM reached 8.46 ± 1.38 ng/ml in rats administered 4.0 g/kg YKS while the C_{max} of GA was 839 ± 112 ng/ml after administration of 1.0 g/kg YKS. The time to maximum drug concentration (t_{max}) was 1.2 ± 0.6 h for GM and 8.7 ± 0.7 h for GA, at which time both ingredients were detected in the brain (Imamura et al., 2011; Tabuchi et al., 2012; Kushida et al., 2013). *In vitro* BBB permeability studies using co-cultured primary rat brain endothelial cells, pericytes, and astrocytes indicated that GM and GA are able to cross brain endothelial cells (Imamura et al., 2011; Tabuchi et al., 2012). Considering that most glycyrrhizin (a glycoside of GA) is metabolized to GA in the intestine by bacterial flora, after which GA is absorbed into the blood (Akao et al., 1994; Takeda et al., 1996; Ploeger et al., 2001), these results suggest that GM is absorbed directly into the blood or glycyrrhizin is as a metabolite GA, and then reaches the brain through the BBB. More recently, almost equivalent data were obtained in the plasma of healthy Japanese volunteers orally administered YKS in a cross-over, randomized study (Kitagawa et al., 2015). These results are consistent with a previous finding that GA was detectable in the plasma after glycyrrhizin or Glycyrrhiza was orally administered to rats (Wang et al., 1994) and human (Cantelli-Forti et al., 1994).

GM absorbed into the plasma from the intestine is rapidly eliminated with a half-life ($t_{1/2}$) of 1.7 h. Studies using recombinant human cytochrome P450 (CYP) isoforms and human liver microsomes suggest that GM is metabolized into hydroxylated, dehydrogenated, hydroxylated plus dehydrogenated, demethylated, and water adduct forms by CYP isoforms (Kushida et al., 2015; Matsumoto et al., 2016). Among these CYP isoforms, CYP3A4 has been found to mainly contribute to GM metabolism. On the other hand, GA exhibits substantially longer t_{max} (8 h) and $t_{1/2}$ (11 h) values than GM. Glycyrrhizin, an ingredient of Glycyrrhiza, was poorly absorbable and not detected in plasma. Glycyrrhizin is mainly absorbed as its active metabolite GA after presystemic hydrolysis by intestinal bacterial flora in the gastrointestinal tract. GA absorbed into the blood is taken up into the liver by capacity-limited carriers, and metabolized into glucuronide and sulfate conjugates. These conjugates are transported efficiently into the bile. After outflow of the bile into the duodenum, the conjugates are hydrolyzed back to GA by commensal bacteria, which are subsequently reabsorbed, causing a pronounced delay to reach the terminal plasma concentration (Ploeger et al., 2001).

Thus, YKS is a mixture of components, some with relatively short half-lives (1.7 h) and other with relatively long half-lives (11 h). In addition, pharmacokinetic evidence, including measurements of GM and GA in the plasma and brain and of their BBB permeability, supports the possibility that these are the active ingredients conferring the psychotropic effects of YKS.

4.2. Distribution of geissoschizine methyl ether (GM) and 18β-glycyrrhetinic acid (GA) in the brain

In rats, both GM and GA do reach the brain by crossing the BBB following oral YKS administration. Then, to identify and characterize the binding sites of GM and GA in the brain, autoradiography using tritium-labeled GM ([^{3}H]GM) or GA ([^{3}H]GA) was performed in rat brain sections (Mizoguchi et al., 2014a,b). Specific binding of [^{3}H]GM was observed in the frontal cortex, including the PFC (e.g., prelimbic cortex), hippocampus, caudate putamen, amygdala, central medial thalamic nucleus, and dorsal raphe nucleus, regions related to various psychiatric symptoms and critical for learning and memory functions. Specific binding with relatively high affinity was dense in the frontal cortical region, moderate in the dorsal raphe nucleus, and sparse in the cerebellum. The specific binding of [^{3}H]GM was significantly inhibited by the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT, 5-HT$_{2A}$ receptor antagonist ketanserin, 5-HT$_{2B}$ receptor agonist BW723C86, 5-HT$_{2C}$ receptor agonist RO60–0175, adrenergic α_{2A} receptor antagonist yohimbine, L-type Ca^{2+} channel blocker verapamil, and μ-opioid receptor antagonist naloxone in the prelimbic cortex, frontal cortex, and dorsal raphe nucleus. Microautoradiography revealed that [^{3}H]GM signals were distributed throughout the frontal cortex, in which neuron-like large cells were included. These results demonstrate that specific binding sites for GM exist in rat brain and suggest that the pharmacological actions of GM are associated mainly with 5-HT receptors in the frontal cortex and dorsal raphe nucleus (Mizoguchi et al., 2014b) (Fig. 4).

Specific binding of [^{3}H]GA was highest in the hippocampus, moderate in the caudate putamen, nucleus accumbens, amygdala, olfactory bulb, cerebral cortex, thalamus, and mid brain, and lower in the brain stem and cerebellum. Several steroids, gap junction-blocking reagents, glutamate transporter-recognizing compounds, and glutamate receptor agonists did not inhibit [^{3}H]GA binding. Microautoradiography showed that [^{3}H]GA signals in the hippocampus were distributed in small non-neuronal cells resembling astrocytes. Immunohistochemical analysis revealed that immunoreactivity for 11β-hydroxysteroid dehydrogenase type-1, a defined molecule recognized by GA (Irie et al., 1992), was detected intensely in neurons, moderately in astrocytes, and very slightly in microglial cells of the hippocampus. These results demonstrate that specific binding sites for GA exist in rat brain and suggest that the pharmacological actions of GA may be related

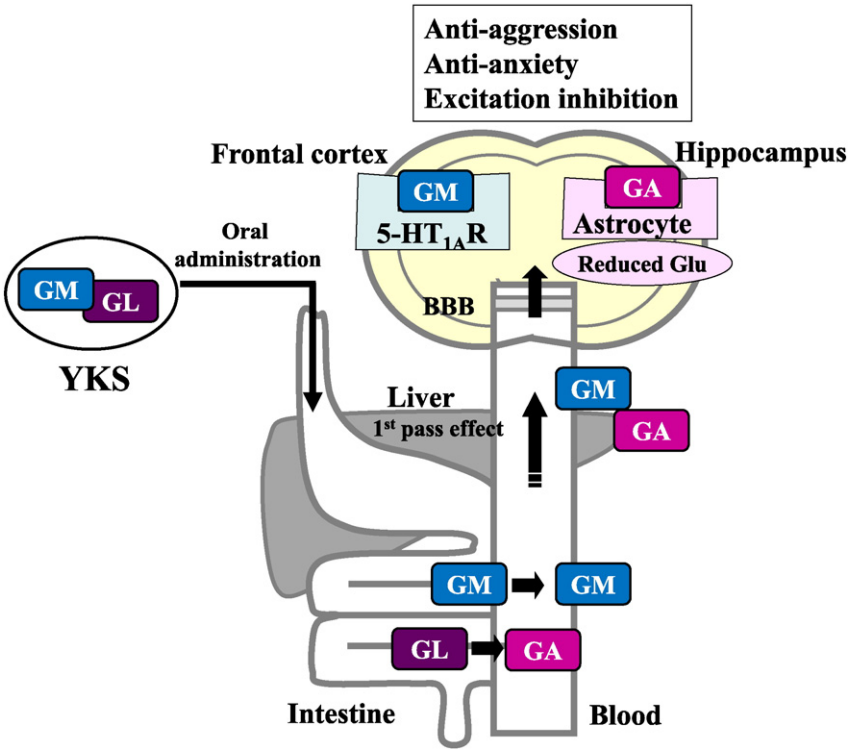

Fig. 4. Possible mechanisms underlying the neuropharmacological efficacy of YKS through GM and GA. After oral YKS administration, glycyrrhizin (GL) is metabolized to GA by intestinal flora. Then, unmetabolized GM and GA are absorbed into blood, and reach the brain by crossing the BBB. In the brain, it is possible that GM predominantly binds 5-HT_{1A} receptors in the frontal cortex as the partial agonist, which mediates anti-aggressive and anxiolytic effects, and GA distributes to astrocytes in the hippocampus, which reduces excessive glutamate (Glu) and inhibits excitation.

to 11β-hydroxysteroid dehydrogenase type-1 in astrocytes (Mizoguchi et al., 2014a) (Fig. 4).

5. Conclusion

Aging is one of the contemporary social issues, and dementia is one of the typical diseases associated with age. In Japan, the current number of patients with dementia has exceeded 460 million people. That is, one in four people older than 65 years of age has some form of dementia or mild cognitive impairment. Cognitive deficits and BPSD are typical features of AD, DLB, vascular dementia, and other forms of senile dementia. Clinical studies have demonstrated that YKS ameliorates various BPSD, including agitation/aggression, irritability, hallucinations, anxiety, sleep disturbance, and aberrant motor activity, as well as other symptoms including pain and itching.

In this review, we focused on evidence obtained from preclinical basic studies demonstrating the neuropharmacological efficacy of YKS, its bioactive ingredients, and pharmacokinetics. As concluded in Fig. 2, YKS had multiple neuropharmacological actions. Studies to identify the active ingredient suggested that alkaloids in Uncaria hook and triterpenes and flavonoids in Glycyrrhiza were major contributors to the neuropharmacological actions of YKS (Fig. 3). Among them, it was found that GM and GA are particularly important bioactive ingredients in the serotonergic and glutamatergic mechanisms, respectively. Referring the pharmacokinetic findings, these ingredients reached the brain, in which GM might ameliorate aggressiveness and anxiety through partial agonistic actions for 5-HT_{1A} receptors in the frontal cortex and GA might reduce excessive glutamate by acting glutamate transporters in astrocytes (Fig. 4).

Nonetheless, YKS is composed of seven dried medicinal herbs containing a myriad of chemical ingredients. Although speculative, the formula of YKS might be modified to express multiple neuropharmacological actions that are integrated to treat neurosis and insomnia without severe side effects, thereby maximizing the clinical benefits. Generally, traditional herbal medicines are a mixture of several crude drugs and consist of a large number of components or ingredients, which may systemically control a complex network system formed by numerous biomolecules. Such medicines are accordance with a theory of multicomponent drugs (Kitano, 2007), and the broad efficacy of YKS is consistent with this theory. In addition, traditional herbal medicines could be considered as a 'long-tail' drug because many of the ingredients are thought to be on the long tail of a statistical distribution in the formula (Kitano, 2007). Although we identified GM and GA as main contributors for the psychotropic actions of YKS, the intrinsic benefits of YKS are probably attributable to the complex activities of the whole formula rather than individual components or ingredients. This idea is supported by the finding that the United States National Cancer Institute identified only 3 effective drugs in 35,000 samples of roots and fruits from 12,000 plant species (Yuan & Lin, 2000), suggesting that additive and/or synergistic effects of multiple components may be associated with pharmacological actions of natural plants, including Kampo medicine. The information described in this review could facilitate to understand the pharmacology of multicomponent drugs, and may also be available to develop therapeutic drugs for dementia based on a systems-oriented drug design approach (Kitano, 2007).

More recently, the safety and frequency of adverse drug reactions in 3156 patients treated with YKS were investigated for up to 52 weeks (Hisada et al., 2014). Adverse drug reactions occurred in 136 cases (4.3%) after the administration of YKS. Major adverse drug reactions were metabolism and nutrition disorders, with hypokalemia being the main symptom (1.4%), general disorder and administration site condition (1.0%), and gastrointestinal disorder with diarrhea and nausea as the chief symptoms (0.6%). Although these incidences are relatively low, since YKS contains Glycyrrhiza, hypokalemia should be closely monitored. For more widespread clinical application of YKS, it is

necessary to accumulate more safety information and side effect profiles in addition to further evidence on the effectiveness, molecular mechanisms of bioactive ingredients, and their interaction.

Conflict of interest statement

The authors are employees of Tsumura & Co. The authors declare that, except for income received from the employer, no financial support or compensation has been received from any individual or corporate entity and no conflict of interest exists.

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