1 **Rapid antidepressant effects of Yueju: A new look at the**
2 **function and mechanism of an old herbal medicine**

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Abstract

Ethnopharmacological relevance: Yueju is a traditional herbal medicine which consists of five herbs and formulated to treat depression-related syndromes 800 years ago. Yueju is still widely prescribed to treat conditions which include digestive dysfunction and depression. Recently, Yueju has been shown to promote a fast-onset antidepressant effect clinically and in preclinical studies. Because conventional antidepressants have a delayed onset in treating depression, the novelty of Yueju's rapid antidepressant effect and its underlying mechanism are of great significance both clinically and scientifically.

Aim of the study: To review the use of Yueju for treatment of mood-related syndromes, and particularly its use in depression. To evaluate recent evidence of Yueju rapid antidepressant actions, based on new findings at behavioral and molecular levels. To suggest direction for future studies to address further scientific issues.

Materials and methods: Reports regarding to the history and current use of Yueju are summarized. Recent progress on rapid antidepressant effects of Yueju, the crucial constituent, *Gardenia jasminoides* J.Ellis (GJ) and other herbs, are reviewed.

Results: The medical need for rapid antidepressant actions, as well as breakthrough findings using ketamine and its limitations are introduced. Studies with Yueju using a number of acute, subacute and chronic behavioral paradigms are compared with ketamine. Findings from clinical reports also support the rapid action of Yueju. Studies examine the contribution of the constituent herb GJ, in rapid antidepressant effects. Importantly, research into the mechanism of Yueju or GJ's antidepressant response indicate the importance of up-regulation in the neural circuit responsible for antidepressant activity, and highlight common and specific molecular signaling by Yueju that may explain why this herb formula has unique antidepressant activity.

Conclusion: Preclinical and clinical studies demonstrate that Yueju confers rapid antidepressant effects. The common mechanisms shared both for ketamine and Yueju, as well as the novel mechanism specific to Yueju are examined. Yueju and GJ may have great clinic applicability and further more detailed studies are warranted .

Keyword: Yueju pill, rapid antidepressant, *Gardenia jasminoides* J.Ellis, BDNF,

Introduction

Major depressive disorder (MDD) is a devastating psychiatric disorder that has substantial effects on the quality of life of patients and their families. The essential features of major depressive disorder include a period of at least 2 weeks during which patients exhibit depressed mood or a complete loss of interest or pleasure in nearly all activities (Battle, 2013). Depression is ultimately related to shorter life spans and higher risk of heart disease (Kessler, 2012). The prevalence of MDD is widespread among world populations, making it one of the leading causes of global disability and socioeconomic burden (Yang et al., 2015). Four hundred and fifty million depressed patients worldwide (Kiyohara and Yoshimasu, 2009), especially those who are at risk for suicide, require an effective, fast-onset antidepressant treatment (Scheuing et al., 2015).

Unfortunately, current conventional antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), only have moderate rates of response and remission, and have unwanted side effects (Kogoj, 2014; Lane, 1998; Levinstein and Samuels, 2014). Moreover, it takes several weeks before SSRIs have therapeutic efficacy. This is especially dangerous to patients at high risk for suicide (Brunoni et al., 2010; Fava, 2003). SSRIs increase the extracellular level of the neurotransmitter serotonin by limiting its presynaptic reabsorption and increasing the level of serotonin in the synaptic cleft available to bind to postsynaptic receptors. The increase of serotonin, or other monoamines, has long been postulated to be a key mechanism underlying antidepressant effects. However, it does not explain the delayed in onset for conventional antidepressants or treatment-resistant depression. Thus, the development of efficacious, fast-acting antidepressants is urgently needed.

Recently, ketamine, an NMDAR (N-methyl-D-aspartate receptor) antagonist, caught widespread attention because of its rapid antidepressant effect. A single dose of ketamine resulted in antidepressant effects which occur as fast as 2 h post-drug, and persisted for up to 7 days in clinical (MDD patients) and preclinical models of depression (Li et al., 2010; Zarate et al., 2006). Unfortunately, the psychotomimetic properties, abuse potential and other side effects of ketamine necessitate caution in its

widespread clinical use (Kalsi et al., 2011; Wang et al., 2013). Therefore, it is often used as a prototype and a number of compounds with the rapid antidepressant potential have been investigated, but few of them have been tested clinically. In Traditional Chinese Medicine (TCM), there is a long history of treating mood disorders with herbal concoctions, acupuncture or other therapeutic approaches, some of which are still actively used. Yueju pill, an herbal medicine formulated ~800 years ago, has recently been found to promote fast-onset antidepressant effects, offering a new avenue to treat depression. As studies begin to shed light on the antidepressant actions of TCM or natural medicines, here we review the progress on preclinical and clinical research into Yueju's antidepressant effects and the underlying molecular mechanisms, in comparison to ketamine. We also examine some of the crucial phytochemical substrates in Yueju that may be responsible for its antidepressant effects.

The clinical use of Yueju pill in Traditional Chinese Medicine

Yueju pill was formulated by TCM theoretician and Master Doctor, Zhu Danxi to treat disorders originating from Yu (Stagnation). He theorized that there were six types of Yu, including stagnation of Qi, Blood, Fire, Dampness, Feeding and Phlegm, which could all be induced by dysregulation of the stress response, and together contributed to the progression of disorders in the mood and body. Different types of Yu syndrome is manifested with a broad collection of symptoms, including depressed mood, chest pain, irritability, chest stuffiness, or feeling blockage in the throat etc., many of which are shared with clinical MDD. Dr. Zhu used five herbs that individually dealt with the different types of Yu, including Xiang Fu (*Cyperus rotundus* L., Cyperaceae.) for Qi Yu, Chuan Xiong (*Ligusticum striatum* DC., Apiaceae) for Blood Yu, Zhi Zi (*Gardenia jasminoides* J.Ellis, Rubiaceae) for Fire Yu, Cang Zu (*Atractylodes lancea* (Thunb.) DC., Compositae.) For Dampness and Phlegm Yu, and Shen Qu (Massa Medicata Fermentata) for Feeding Yu. Yueju is made with the mixture of these five dry herbs. Yueju was broadly used to treat diseases that show signs of the six types of Yu historically, and it is still used today. In TCM, Yueju is

more often prescribed to treat digestive system diseases, including esophageal disease, indigestion, stomach diseases, intestinal diseases, peptic ulcer, liver disease, gallbladder disease, and pancreatic disease. It is also used as a primary or supplementary part of a prescription to treat cardiovascular and respiratory disease conditions.

As symptoms of Yu overlap in many aspects with mental disorders, nowadays Yueju is also prescribed for treatment of depression. A number of clinical observational or hypothesis-driven studies have reported an antidepressant effect in Chinese literature. Based on these reports, depression can be ameliorated by repeated oral Yueju administration, either as a monotherapy or adjunct with conventional antidepressants. There were more than 10 studies showing that Yueju is effective in treating various types of depression, including post-stroke depression, postpartum depression, and depression in seniors. Many reports concerned its use post-stroke depression treatment, because patients suffering from post-stroke depression typically exhibit symptoms of Blood Yu, Fire Yu, and Phlegm Yu. However, rigorous studies to establish the antidepressant efficacy of Yueju remain to be done.

Preclinical and clinical evidence of rapid antidepressant-like behavioral effects of Yueju

The rapid antidepressant effects of Yueju have been revealed both experimentally and clinically. Additionally, it was found that only the constituent herb *Gardenia jasminoides* J.Ellis displayed rapid antidepressant potential. The findings are summarized in the Table1 below. Four features should be noted: 1) All the behavior in acute or chronic models were tested after a single dose; 2) Protracted antidepressant effects after a single dose of Yueju were characterized in different models; 3) Different strains of mice have been tested to investigate the genetic impacts on the antidepressant effects of Yueju; 4) Ketamine was used as a positive control for fast-onset of antidepressant effects.

References	Species strains	Behavioral paradigms	Behavioral effects	Neuromolecular mechanism
Xue et al., 2013	ICR mice	learned helplessness (LH) and tail suspension test (TST)	Effective on LH at day 1; on TST from hour 0.5 to day 1 post Yueju	Rapid promotion of hippocampal BDNF expression likely by protein synthesis through deactivation of eEF2
Xue et al., 2016	Kunming and ICR mice	TST	Effective on TST from hour 0.5 to day 5 in Kunming mice and from hour 0.5 to day 1 in ICR mice post Yueju	Rapid promotion of hippocampal BDNF protein expression, followed by persistent activation of PKA-CREB and BDNF mRNA expression in Kunming mice. No PKA-CREB activation in ICR.
Tang et al., 2015	Kunming mice	sucrose preference test (SPT), TST, forced swim test (FST), and novelty-suppressed feeding test (NSF)	In mice subjected to chronic mild stress, effective on SPT from hour 2 to day 6; on TST, FST and NSF from day 1 to day 5, and on body weight gain at day 6 post Yueju.	Increased prefrontal mTOR signaling, p-ERK p-Akt, and synaptic proteins GluR1, PSD95, and synapsin 1, and downregulated NR1 expression .
Xia et al., 2016	Kunming mice	FST and LH	Effective on FST from hour 0.5 to day 5, on LH at day 3 post Yueju.	Rapid reducing NR1 expression and sustaining for 5 days in the hippocampus.
Zhang et al., 2015	Kunming mice	TST, LH and NSF	Effective TST from hours 2 to day 1 for Gardenia jasminoides Ellis (GJ), on LH at day 1 post GJ, on NSF at day 1 post GJ or Yueju.	BDNF expression in the hippocampus was up-regulated from 2 hours, persistent over 20 hours.
Tao et al., 2014	Kunming mice	TST and FST	Effective on TST and FST at day 1 post CO ₂ Fraction of GJ.	
Wu et al., 2016	Kunming mice	TST and LH	Effective on TST from hour 0.5 to day 3 post yellow	30 minutes post GYP administration, ERK signaling was activated, inhibiting downstream effector p-eEF2,

			pigment of GJ (GYP), on LH at day 1 post GYP.	up-regulating expression of GluR1 and Synapsin 1. CREB signaling and BDNF expression were up-regulated at day 1.
Ren et al., 2016	Kunming mice	TST, FST, SPT and NSF	In mice subjected to chronic mild stress, effective on TST, FST, SPT and NSF at 1 day post PE or BO fraction of GJ.	PE, but not BO fraction of GJ showed a significant increase in the expression of BDNF and TrkB in the hippocampus, from 2 hours to day 1.
Xia et al., 2016	Balb/cJ mice	SPT, FST and NSF	In postpartum depression-like mice, effective on SPT, FST and NSF from day 1 to day 5 post Yueju.	

Table1. Summary of rapid antidepressant effects of Yueju

Abbreviations: BDNF, Brain-derived neurotrophic factor; eEF2, eukaryotic elongation factor 2; p-eEF2, Phosphorylated eukaryotic elongation factor 2; PKA, Protein kinase A; CREB, cAMP-response element binding protein; mTOR, Mammalian target of rapamycin; p-ERK, Phosphorylated extracellular regulated protein kinases; p-Akt, Phosphorylated protein kinase B; TrkB, Tyrosine kinase receptor B; GluR1, α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor 1; PSD95, Postsynaptic density protein 95; NR1, NMDA receptor 1; PE, Petroleum ether; BO, N-butyl alcohol.

Rapid antidepressant potential of Yueju in alleviating behavioral despair and anxiety-like behavior

Yueju was examined for effects on behavioral despair in mice in response to acute stress, using the tail suspension test (TST) and forced swim test (FST). In both tests, an animal is exposed to a short term stress that they cannot escape. Initially, the animal struggles, and periodically becomes immobile, before eventually giving up (Cryan and Holmes, 2005). The immobility time is considered an indication of behavioral despair and antidepressants typically reduce immobility time in the TST and FST (Browne and Lucki, 2013). Although some SSRIs reduce immobility time,

the effect of a single dose of ketamine is both immediate and persistent, which is not evidenced using conventional antidepressants (Duman et al., 2012). By taking advantage of this property, potential fast-onset antidepressant drugs can be screened by testing for an antidepressant response in mice using the TST or FST after a single dose. In ICR strain mice, different doses of ethanol extracted Yueju were tested, but only the 1400mg/kg dose showed a protracted antidepressant effect. There were no effects at lower (700mg/kg) or higher (2800mg/kg) doses. The effective dose also reduced immobility time in TST or FST immediately (thirty minutes after a single administration) (Xue et al., 2013). The antidepressant activity of Yueju or ketamine lasted for 5 days in Kunming strain mice (Tang et al., 2015; Xia et al., 2016; Xue et al., 2016). These observations suggest that Yueju has the rapid antidepressant potential, laying the foundation for collecting more evidence from other behavioral tests to assess rapid antidepressant efficacy.

Antidepressants are also used in treating anxiety, and anxiety is frequently comorbid with clinical depression. Therefore, some anxiety-like paradigms were also used for testing rapid antidepressant potential. Hyponeophagia, one of the anxiety-like paradigms, is defined as a reduction in feeding in response to novelty in rodents. Novelty suppressed feeding (NSF) tests hyponeophagia in an unfamiliar environment. A longer latency or reduced food consumption indicates an anxiety-like response (Dulawa and Hen, 2005). Antidepressants can reverse this response, however conventional antidepressants need to be administered repeatedly (Banasr et al., 2007). A single dose of Yueju, similar to ketamine, reduced the latency to feed and increased food consumption in NSF (Zhang et al., 2015). It is worth noting that the effective dose of Yueju did not alter the locomotor activity in the open field test, showing that anxiety-like behavioral testing was not confounded by locomotor effects. In fact, neither Yueju nor ketamine altered anxiety-like behavior in the open field test (Xue et al., 2013).

Rapid antidepressant response of Yueju in alleviating learned helplessness

Learned helplessness (LH) is a validated animal model of depression (Miller et al.,

1975; Seligman and Beagley, 1975; Seligman et al., 1968). LH occurs when an animal is repeatedly subjected to an aversive stimulus (normally foot shock) from which it cannot escape. Eventually, the animal stops trying to avoid the adverse stimulus. This response was first demonstrated in animals experimentally, but has since been identified in some subpopulations of MDD patients (Maier and Watkins, 2005). Repeated administration of the conventional SSRIs is required to elicit an effect on LH (Caldarone et al., 2000; Shanks and Anisman, 1988). In contrast, in mice a significant reduction in LH was detected 1 day after a single administration of Yueju or ketamine. Additionally, the effects of Yueju on LH were observed for at least 3 days after a single dose (Xia et al., 2016). Thus, the evidence from LH paradigm also supports rapid and long-lasting antidepressant effects of Yueju.

Rapid antidepressant response of Yueju in reversing depression-like behavior in chronically stressed animals

Chronic Mild Stress (CMS) is a universally recognized animal model that mimics the clinical depression. CMS models induce depression-like behavior in rodents following the presentation of either same stressor (such as restraint) or a series of stressors in an unpredictable sequence over a prolonged period of time. Also similar to clinical observations, behavioral and molecular changes induced by CMS are reversed by treatment with SSRIs, but only after administration for several weeks (Browne and Lucki, 2013), thus CMS is useful for testing rapid antidepressant effects. A core symptom of depression, anhedonia, is quantified with a reduction in preference to sweet fluids following CMS (Zeng et al., 2014). The rapid antidepressant effect of Yueju was assessed using a chronic unpredictable mild stress protocol (Tang et al., 2015). Both Yueju and ketamine restored sucrose preference impaired by CMS within 2 hours. This is the first experimental demonstration showing an early onset of antidepressant effect using this model. As predicted, a single dose of fluoxetine had no effect on sucrose preference. Additionally, Yueju also alleviated other signs of depression in animals subjected to CMS. These include reduction of immobility time in TST or FST, and decreased latency for eating and increased food consumption in

the NSF test. Interestingly, a single administration of Yueju improved body weight gains more significantly than ketamine. Reduced body weight is an important clinical symptom of depression, and a rapid improvement in weight gain in preclinical models has not been reported previously. Furthermore, Yueju showed a more persistent effect in SPT and NSF than ketamine in CMS mice (Tang et al., 2015). All together these findings from the CMS model of clinical depression support the rapid antidepressant efficacy of Yueju using multiple measurements at variable time points.

Fast-onset antidepressant effects of Yueju in MDD patients

A clinical investigation was carried out to test rapid antidepressant effects in MDD patients who have not previously been treated with antidepressants. In this pilot random, double-blinded, and placebo-controlled clinical study, the antidepressant effect during the first 7 days of treatment using a combination of Yueju with the widely used SSRI, fluoxetine (experimental group), was compared to a combination of an herbal placebo with fluoxetine (control group). As predicted, there was no change in standard depression scores in the control group during the first 7 days of treatment. In contrast, beginning at 3 days, there was a significant improvement in the alleviation of depression symptoms in experimental group, when compared to their baseline levels and compared to the control group. Additionally, there was a significantly higher response rate in the experimental treatment group. The rapid antidepressant effects were not induced by a prior treatment. Adverse effects, found equally in both groups included diarrhea and constipation (Wu et al., 2015). The results indicate that Yueju is promising in rapidly alleviating depressive symptoms. However, more studies are required to assess potential side effects of Yueju, and to assess Yueju monotherapy of Yueju on a larger sample of MDD patients.

***Gardenia jasminoides* J.Ellis and effective ingredients for rapid antidepressant effects of Yueju**

Gardenia jasminoides J.Ellis (GJ) was found to be the only constituent herb in Yueju that conferred rapid-antidepressant response (Zhang et al., 2015). The dose of

the ethanol extract of GJ (420 mg/kg) that elicited a rapid antidepressant potential was modestly higher than the proportion of GJ (280 mg/kg) in Yueju. There was no effect using a higher GJ dose (560mg/kg). This is similar to the dose-effect pattern seen with Yueju. In contrast, the other four constituent herbs in Yueju didn't show any antidepressant effects at either proportional or modestly higher doses. The effective dose of GJ also rapidly alleviated deficits LH and NSF paradigms. The antidepressant response of GJ in TST started 2 hours after administration, suggesting a slightly later onset than Yueju. The higher dose needed and delayed onset with GJ suggests that other constituent components in Yueju play supporting roles in accelerating or enhancing GJ's antidepressant effects. Studies further identified that the petroleum ether fraction (GJ-PE), or n-butyl alcohol fraction (GJ-BO), may contain the compounds responsible for the rapid antidepressant efficacy of GJ. Acute administration of either GJ-PE or GJ-BO significantly reversed the behavioral deficits in the tests of TST, FST, SPT and NSF in chronically stressed mice (Ren et al., 2016; Tao et al., 2014). One of the major bioactive components, gardenia yellow pigment, which is a collection of compounds with a shared crocin structure, is enriched in the GJ-BO fraction, and also induces rapid and persistent antidepressant responses in mice (Wu et al., 2016), although a higher dose is required. Preclinical and clinical studies indicate that chronical administration of crocin or gardenposide alleviated depression behaviors or symptoms, but their contribution to rapid antidepressant effects of Yueju remains to be further established.

Neural molecular mechanisms underlying rapid antidepressant effects of Yueju

Up to now, cellular and molecular underpinnings for rapid antidepressants were mostly based on the studies using ketamine. Ketamine's antidepressant actions are dependent on glutamate stimulation of brain-derived neurotrophic factor (BDNF) release followed by intracellular Akt-mTOR signaling activation and spinal formation (Autry et al., 2011; Li et al., 2010). The antidepressant effects of Yueju are similar to ketamine, with involvement of NMDA receptors, APMA receptors, BDNF and Akt-mTOR pathways (Tang et al., 2015). However, other studies have revealed the

more importance role of PKA-CREB signaling in antidepressant effects of Yueju (Xue et al., 2016).

Yueju rapidly increases BDNF expression in the hippocampus

BDNF and its high affinity for tyrosine kinase receptor B (TrkB) activate a number of signaling pathways which regulate neuronal growth and survival (Browne and Lucki, 2013). BDNF has been implicated in the pathology of MDD and its treatment (Dwivedi, 2009; Neto et al., 2011; Taliaz et al., 2010). Studies have shown that ketamine-induced synaptogenesis involves the inhibition of the eukaryotic elongation factor 2 (eEF2) kinase, leading to reduced eEF2 phosphorylation and subsequently increased BDNF mRNA translation which is suggested to play an important role in regulating the rapid antidepressant effects of ketamine (Autry et al., 2011). Like ketamine, Yueju also quickly reduces phosphorylation of eEF2 and increases expression of BDNF in the hippocampus of ICR strain mice, leading to desuppression of BDNF synthesis even without change of BDNF mRNA, at 30 minutes after a single dose of administration. Although Yueju still showed antidepressant effects, the expression of BDNF was reduced. It seems that the increase of BDNF protein expression is crucial for development of immediate antidepressant response but may not be involved in the late stage antidepressant effects in ICR strain (Xue et al., 2013). Up-regulation of hippocampal BDNF expression is temporally linked to the onset of antidepressant effect of GJ (Zhang et al., 2015). Interestingly, rapid antidepressant effects of GJ-PE were associated with up-regulated BDNF and TrkB expression. However, rapid antidepressant effects of GJ-BO as well as its subfraction component GPY were relatively independent of BDNF signaling, whereas BDNF increased only at later time by GPY (Ren et al., 2016; Wu et al., 2016).

Acute Yueju reversed the deficient in Akt-mTOR signaling in the prefrontal cortex of chronically stressed animals

Activation of mammalian target of rapamycin (mTOR) signaling (in PFC) is

suggested to be crucial for rapid antidepressant effects of ketamine or other rapid antidepressant-like drugs (Tang et al., 2015). Activation of mTOR-related signaling pathways may also be involved in the fast-onset antidepressant effects of Yueju (Tang et al., 2015). Although CMS did not affect mTOR activation, it decreased phosphorylation of the mTOR effectors 4E-BP1 and p70S6K, mTOR upstream regulators ERK and Akt, and downstream targets including synaptic protein GluR1 (Glutamate receptor 1, an AMPA receptor subunit). A single dose of Yueju normalized all of these changes. Furthermore, Yueju, but not ketamine, maintained the reversal of Akt signaling deficiency 6 days post-administration, indicating a temporal association of Akt signaling with antidepressant efficacy (Tang et al., 2015). Further studies showed that blockade of mTOR signaling blunted antidepressant effects and up-regulation of BDNF gene expression by ketamine, but not Yueju, suggesting the relative independence on mTOR signaling for Yueju's antidepressant efficacy (Xue et al., 2016). The role of Akt signaling in Yueju's antidepressant effects should be clarified by further study.

Down-regulation of NR1 Expression was associated with antidepressant effects of Yueju

NMDA receptors consist of the obligatory subunit NR1 and various modulatory subunits (e.g., NR2A-D subunits). Chronic stress in mice induces a selective and long-lasting increase in NR1 expression in the PFC, which is attenuated by Yueju. There was an even longer attenuation of NR1 expression by Yueju than ketamine, which goes in parallel with the longer duration of the effects of Yueju (Tang et al., 2015). In an animal model of LH, the expression of NR1 subunit in the hippocampus is increased. This is sustained for several days after the stress, whereas a single dose of Yueju reverses this effect (Xia et al., 2016). In comparison, Yueju only induces an immediate but not a lasting inhibition of the expression of NR2B, and does not alter NR2A expression (Xia et al., 2016). These findings support the role of NR1 in the hippocampus with Yueju's rapid and long-lasting antidepressant action. Additionally, the long-lasting antidepressant effects of Yueju are not associated with changes in

AMPA subunit expression (Tang et al., 2015). Unlike ketamine, blockade of transmission did not affect the antidepressant effects of Yueju (Xia et al., 2016).

Activation of PKA-CREB signaling pathway was responsible for long lasting antidepressant activities of Yueju.

CREB (cAMP-response element binding protein) signaling in the hippocampus has been implicated in affective and cognitive behaviors. CREB activation is a hallmark of the neural plasticity responsible for antidepressant effects after chronic SSRI, and a novel role of this signaling in rapid antidepressant effects was detected based on effects of Yueju (Xue et al., 2016). There was long-lasting increase in CREB gene expression after Yueju, and enhanced phosphorylated CREB (pCREB) signaling in Kunming mice with a lasting antidepressant effect. This is in contrast to a lack of changes in CREB signaling in ICR mice. ICR mice show a much shorter duration of antidepressant response to Yueju. PKA, the upstream regulator of CREB, was also activated in Kunming mice. Both strains showed rapid antidepressant effects after Yueju's administration. These results indicate that CREB signaling was responsible for enduring, but not immediate antidepressant responses. Importantly, inhibition of PKA-CREB signaling blunts antidepressant effects and also blocks up-regulation of BDNF gene expression by Yueju, but not by ketamine. This indicates a specific role of this signaling pathway in the effect of Yueju (Xue et al., 2016). A delayed up-regulation of BDNF and CREB were also demonstrated following administration of GYP. Activation of CREB and BDNF expression after GYP administration is thus suggested to be involved in persistent antidepressant activity (Wu et al., 2016). On the basis of existing information, a framework of the rapid antidepressant mechanism of Yueju is proposed and illustrated in Figure 1.

Figure 1. Signaling pathway underling Yueju's rapid antidepressant effects.

A single dose of Yueju persistently inhibits the activity of NMDAR, NR1, and subsequently activates the AKT and Erk signaling, leading to activation of p70S6K and 4EBP, downstream effectors mTOR signaling, leading to synaptic protein synthesis, including AMPAR. The activated ERK also suppressed the phosphorylation of eEF2, which increases the synthesis of

proteins, including BDNF. This activation pattern is similar to ketamine. A specific signaling pathway by a single dose of Yueju is PKA-CREB-BDNF pathway, which can be activated by PACAP and promotes neural plasticity responsible for antidepressant activities.

Conclusion

Together, findings from acute, subacute and chronic behavioral models all support the rapid antidepressant effects of Yueju, an herbal medicine previously used for treatment of Yu syndromes, many symptoms of which are shared with clinical depression. Yueju even shows greater antidepressant efficacy on some key tests than ketamine. Its fast-onset antidepressant response was also supported by the findings from a pilot random controlled trial. Regarding the neurobiological mechanism, Yueju, like ketamine, rapidly increases the expression of BDNF in the hippocampus, via nontranscriptional modification, which may be crucial for the fast antidepressant response to Yueju. Similar to ketamine, in both the chronic stress and LH conditions, a single dose of Yueju normalized up-regulation of NR1 mediated by depression-like symptoms, suggesting the interference with NMDA receptor NR1 subunit function may be mechanically linked to the antidepressant activity of Yueju. Both Yueju and ketamine also reverse deficits in Akt-mTOR related signaling, synaptic protein synthesis and the imbalance of AMPA/NMDA receptor ratio after chronic stress, although mTOR signaling or AMPA receptor itself were not required for the action of Yueju, unlike ketamine. Instead, activation of PKA/CREB pathway and subsequent induction of transcriptional BDNF mRNA expression was responsible for the long lasting antidepressant effects specifically for Yueju. The constituent herb *Gardenia jasminoides* J.Ellis has been identified as essential for rapid antidepressant effects of Yueju, and it remains to be determined whether multiple fractions or compounds have different mechanisms.

There are still limitations and gaps in knowledge of Yueju's rapid antidepressant effects. Behaviorally, a few mouse strains, but not rat strains, have been tested. In the tests of NSF, TST, FST, and LH, ketamine was used as a positive control, but it might be more informative if a negative control for fast onset antidepressant effects, such as

an SSRI, were used. Clinical investigations on the rapid antidepressant effects by the single treatment of Yueju is still required. The individual compounds from several identified fractions of Yueju, such as GYP should be isolated and tested for function and mechanism of action. The specific extracellular signaling that stimulates PKA-CREB signaling is also important for establishing potential novel mechanisms underlying rapid antidepressant effects.

In the future, clinical studies with larger sample sizes and more measurements should be performed to provide evidence of the rapid antidepressant action and elucidate side-effects of Yueju. Future studies should address more detailed information of the chemical substrates, the interaction pattern and neuromolecular mechanisms of Yueju. The main clinical use of Yueju was in treatment of gastrointestinal diseases. However, recent evidence suggests that Yueju has pre-clinical and clinical antidepressant effects. Emerging evidence suggests the intrinsic relationship between depression, inflammation and intestinal microbiota (Burokas et al., 2017). This is consistent with the idea that depression itself is not a purely mental disorder; rather it has deep connection with physiological balance in the body. The holistic approaches using herb mixtures such as Yueju that concern both brain and the body may be more promising for improved treatment outcome of MDD.

Conflict of interest

The authors declare that they have no competing financial interests.

Acknowledgments

The study was supported by the National Science Foundation of China (81673625), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). The study was also supported by the Natural Science Foundation of Jiangsu Province (BK20140962, BK20151568 and BK20161040) and the Collegiate Natural Science Foundation of Jiangsu Province (15KJB180011). We are grateful to Dr. Laura Kozell for helpful discussions and comments on a draft of this manuscript.

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