

Original Article

Peony reverses tau phosphorylation level in mouse depression model via BDNF-Akt-GSK3 beta pathway

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Abstract: A number of clinical investigations have shown that depression is a moderate to high-risk factor for Alzheimer's Disease (AD) and 30-50% of depressive patients develop mild cognitive impairment and AD. Although a Chinese herbal formulae peony has significant effects on the treatment of depression-like disorders, whether it could retard the progress and development of AD is unknown. In present study, we established a mouse depression model by repeating injection of corticosterone (CORT). After 3-weeks' injection, mice exerted depression-like behavior, as indicated by significant decreased time in center zone in open field test and increased immobility time in tail suspension test. Increased hyperphosphorylation level of tau and decreased phosphorylation level of Ser9-phosphorylated GSK 3 β (inactive form of GSK 3 β) were observed in hippocampus of depression mouse, and mice's capacity of learning and memory was also impaired. After treating with peony, depressive mice performed much better in morris water maze, as indicated by significant decrease latency time and increase platform crossing times. Hyperphosphorylated level of tau and phosphorylation of GSK 3 β were also reversed. Moreover, it was found that protein level of brain-derived neurotrophic factor (BDNF) in the hippocampus was significantly increased in peony-treated depressive mice compared to CORT-treated mice. This study suggested that peony ameliorate depressive mice's deficiency in learning and memory, attenuate hyperphosphorylation level of tau and activation of GSK 3 β , which is possibly mediated via increasing protein level of BDNF in the hippocampus.

Keywords: Alzheimer's disease, tau, depression, GSK 3 β , peony

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative dementia, affecting nearly 10% of the population over 65 years of age, and this prevalence double every five years after 65 years' old [1]. Aggregation of abnormally phosphorylated tau, one of hallmarks in AD and the main constituent of neurofibrillary tangles, is most likely a key pathological event of AD [2]. After a pooled and meta-regression analyses of 20 selected studies, including more than 100 thousand persons in 8 countries, researchers found that depression is not only a high risk for AD, but also a prodromal symptom. Patients with Alzheimer's show chronically high cortisol levels suggesting changes occurring in controls of hypothalamic-pituitary-adrenal (HPA) axis. Over activity of the HPA axis, elevated cortisol levels are depression symptoms,

which can precede clinical diagnosis of AD for years or occurs around the onset of AD [3], although the etiology and pathologic mechanism of depression in AD pathogenesis are still need explored.

The normal function of tau is to promote microtubule assembly and stabilize the formed microtubules, and thus to establish cellular polarity and maintain the intracellular transport of neurons [4]. When tau is hyperphosphorylated and accumulated in the cells, it becomes incompetent in executing the above biological functions and thus leads to disruption of microtubules [5]. Several studies have shown that chronic stress could increase AD-related markers such as tau phosphorylation and amyloid- β (A β) accumulation in mouse or rats [6-8]. Glycogen synthase kinase-3 β (GSK 3 β) is a key kinase that phosphorylate tau in vitro and in

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vivo and their misregulation is implicated in the formation of neurofibrillary tangles [9, 10]. The level of Ser9-phosphorylated GSK-3 β (inactive form) has been reported significant decrease in a depression rat model, and reversed by selective serotonin reuptake inhibitor (SSRI) citalopram for antidepressant treatment [11]. It's suggested that anti-depressant drug may serve as a potential medicine for the treatment of AD.

The root part of *Paeonia lactiflora* pall, also known as peony, is one of the best-known herbs in China. It has been used in traditional herbal medicine for thousands of years. It is a component herb in herbal formulae (Xiaoyao-san) for treatment of depressive-like disorders in China [12]. Previous reports suggested that total glucosides isolated from the radix of *Paeonia lactiflora* could moderate the activity of hypothalamus-pituitary-adrenal (HPA) axis in rats subjected to heavy stress conditions [13]. Besides from its anti-depressant-like effects, peony also own neuroprotective effects, as it could protect PC12 cells against corticosterone-induced neurotoxicity in vitro [14]. However, how peony influence neuron cells and whether it has any effect on the development and progression of AD is still not known.

The purpose of present study was to study the effects of peony treatment on tau phosphorylation and the behavioral phenotype in 3 weeks' repeated corticosterone injection mice. We examined depressive-like behavior of mice, phosphorylation level of tau and spatial memory. Our results show that repeated injection corticosterone can induce depression-like behavior and tau's hyperphosphorylation and spatial memory deficiency. Then we studied the possible effects of pharmacological intervention in mouse model with peony. We had demonstrated that peony can attenuate tau phosphorylation and spatial memory deficiency induced by repeated injection of corticosterone. Our results support the notion that vulnerability to depression might constitute a risk factor for the development of AD, and peony treatment may represent a therapeutic strategy for the treatment of depressant-like disorder and AD.

Material and methods

Animals

A total of 30 male C57 weighing 20-25 g were obtained from the Center for Experimental

Animals at Wuhan University for use in this experiment. Mice were divided in to three group, control group (VEH, n = 10), CORT group (20 mg/kg subcutaneous injection of corticosterone for 3 weeks, n = 10) and CORT+Pe (20 mg/kg subcutaneous injection of corticosterone and 20 mg/kg subcutaneous injection of corticosterone for 3 weeks, n = 10). All animals were housed in groups of three to four in plastic cages with a controlled environment, a 12:12-h light-dark cycle, and ad libitum food and water for 1 week before the start of the study. The experimental procedures were conducted in conformity with the instructions for the care of laboratory animals issued by the Ministry of Science and Technology of the People's Republic of China in 2006.

Mouse depression model

Two groups (CORT and CORT+Pe group) were administrated subcutaneously with corticosterone (Sigma, 20 mg/kg suspended in physiological normal saline containing 0.1% dimethyl sulfoxide (DMSO) and 0.1% Tween-80) once a day in a volume of 6 ml/kg, while the control group (VEH) was administrated only with the saline. After being administered for 21 consecutive days, the depression-like behavior of the mice was observed in the open field test and the tail suspension test.

Open field test

Motor activity was quantified in four Plexiglas open field box 50 \times 50 cm² (zhenghua, Anhui, China). Two sets of 16 pulse-modulated infrared photobeams on opposite walls 2.5 cm apart recorded x-y ambulatory movements. Activity chambers were computer interfaced for data sampling at 100-ms resolution. The computer defined grid lines that dividing center and surround regions, with the center square consisting of four lines 11 cm from the wall.

Tail suspension test

After the open field test, the mice were allowed to have a rest for 24 h, and then suspended on the edge of a shelf 5 cm above a tabletop by adhesive tape, placed approximately 1 cm from the tip of the tail. They were allowed to hang for 6 min, and the duration of immobility was recorded during the last 4 min of the test. Mice were considered immobile only when they hung passively and completely motionless.

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Morris water maze

The standard Morris water maze (MWM) procedure with minor modifications was used for the spatial training. Briefly, the mice were trained in MWM to find the hidden platform for five consecutive days and four trials per day with a thirty minutes interval. During the training, each mouse was placed into the water by hand, so that it faced the wall of the pool, at one of four starting positions. The animals were not allowed to search the platform for more than 90 seconds, after which they were guided to the platform and placed on the platform for 20 seconds. In each trial, the swimming path and the latency to locate the hidden platform were recorded. At 24 hour after spatial training in MWM, mice were sacrificed to execute the biochemistry experiment. All experiments were conducted and analyzed by the experimenters blind to the grouping of the animals.

Western blot analysis

Mice ($n = 4$) were sacrificed by decapitation while under ether anesthesia. After sacrifice, the brains were rapidly removed from the skulls and the hippocampal tissues were dissected on an ice-cold plate. Then according to our previous procedures [15], in brief, dissected hippocampal tissues were immediately homogenized at 4°C with 0.5 ml of RIPA lysis buffer. Aliquots of the clarified homogenized liquid were denatured in a sample buffer at 95°C for 5 min. The samples were then analyzed by 10% SDS-polyacrylamide gel electrophoresis and transferred to Polyvinylidene Fluoride (0.45 μm PVDF) membranes (Millipore). The primary antibodies included rabbit polyclonal anti-pS396 (1:800), rabbit polyclonal anti-pS404 (1:1000), polyclonal antibody Tau 5 (1:500) were purchased from SAB, China; rabbit polyclonal antibody GSK3 β (1:1000), Ser9-phosphorylated GSK 3 β (1:1000), mouse monoclonal anti-GAPDH (1:3000) were purchased from Cell Signaling Technology. Rabbit polyclonal anti-Akt (1:800), rabbit monoclonal pAkt ser473 (1:1000) were purchased from Abcam. The secondary antibodies were horseradish peroxidase conjugated goat anti-mouse IgG (1:10000, Sigma). Immunoblotting was detected by enhanced chemiluminescence (ECL, Amersham) and analysed using Image J. As for each primary antibody, six animals per group and three protein samples per animal were included in this analysis.

ELISA assay of BDNF and corticosterone

Hippocampus tissue (100 mg $n = 3$) was rinsed with 1 \times PBS, homogenized in modified protein extraction buffer as described [16], followed by BCA quantification assay to determine protein concentration. Hippocampus homogenates BDNF concentration was measured using a commercially available enzyme linked immunosorbent assay kit for BDNF (CUSABIO Life Science Inc, China). This assay employs the competitive inhibition enzyme immunoassay technique. A 96-well immunoplates were pre-coated with monoclonal anti-mouse BDNF antibody. Protein samples (100 μL) was incubated in coated wells at 4°C over night. Then the plates were then incubated with a secondary antibody for 1 hour at room temperature followed by TMB/peroxidase substrate solution and 1 M HCl (100 μL /well). The colorimetric reaction product was measured at 450 nm using a microplate reader. BDNF concentration was determined based on linear regression of the BDNF standards (range = 7.8-500 pg/mL purified mouse BDNF) that were incubated under similar conditions in each assay. For corticosterone assay, serum corticosterone level was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Inc., China) according to the manufacturer's protocol.

Real-time PCR for mRNA of BDNF

Firstly, total RNA was isolated from hippocampus tissue using TRIzol® following manufacturer's recommendations (Life technologies). Briefly, about 40 mg of tissue ($n = 3$) were manually homogenized in 1 mL of TRIzol. The homogenate was centrifuged for 3 minutes at full speed, and the supernatant was transferred to a new tube. A second centrifugation was performed to remove any remaining cellular debris, followed by adding 95% ethanol for RNA precipitation and binding onto the column. The column was centrifuged and washed three times, and the RNA was eluted from the column using RNase-free water. Total RNA concentration was determined at 260 nm/280 nm using NanoDrop Lite spectrophotometer (Thermofish, USA). Total RNA samples were stored at -80°C for further analysis. Complementary DNA (cDNA) synthesis was performed with PrimeScript RT reagent kit (Takara, Japan), using 1 μg of total RNA and oligo dT primers. cDNA was further

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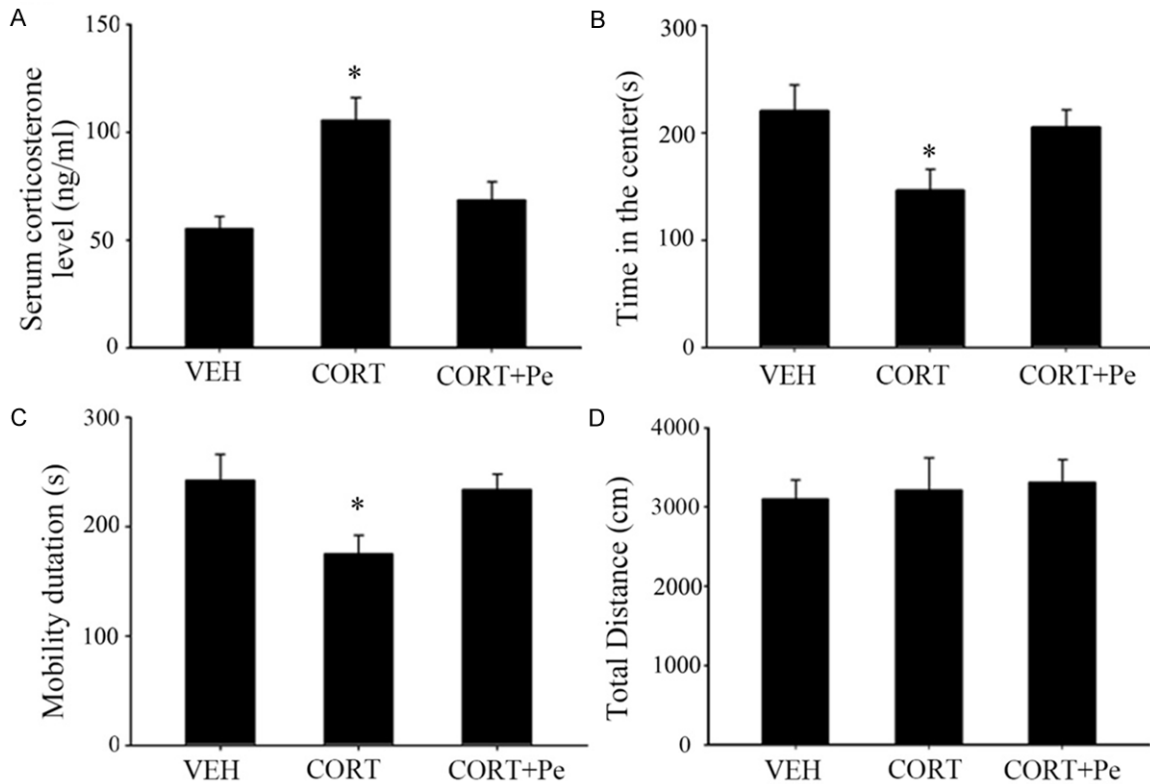


Figure 1. Effects of peony on depression-like behavior of mouse model in the open field test and tail suspension test. (A) After 3 weeks' treatment, CORT concentration in mouse serum was examined by ELISA kit. Mice were placed in a box for 5 minutes, their time spent in the center zone of the box in open field test is shown in (B) and running distance in (D), and the time spent during the last 5 min of tail suspension test is shown in (C). *P<0.05, CORT vs VEH; VEH, vehicle; CORT, corticosterone injection 20 mg/kg; CORT+Pe, corticosterone injection 20 mg/kg and gavage treated of peony at dose of 80 mg/kg.

diluted in deionized water and stored at -20°C . The mRNA expression of BDNF was further assessed by real-time PCR using a One Step SYBR PrimeScript RT-PCR kit (Takara, Japan), with the following forward (F) and reverse (R) primers: (F) 5'-CCATAAGGACGCGGACTTGT-3', (R) 5'-GAGGCTCCAAAGGCACTTGA-3'; and GAPDH (F) TTCAACGGCACAGTCAAG (R) TACTCAGCACCAGCATCA was used as an internal control. Samples were analyzed in triplicate and a melting curve analysis was performed in each sample at the end of RT-PCR reaction. Relative gene expression was determined by $2^{-\Delta\Delta\text{Ct}}$ method. The threshold cycle (Ct) value was determined for target genes and the endogenous internal controls in each sample. The difference between target gene Ct and internal control Ct was determined for each sample, resulting in the ΔCt value. The ΔCt of a calibrator sample was subtracted from each sample ΔCt to yield the $\Delta\Delta\text{Ct}$ value. Relative fold change was calculated as $2^{-\Delta\Delta\text{Ct}}$.

Statistical analysis

The results are reported as means \pm SD. Data were analyzed with a one-way ANOVA followed by the Bonferroni test for post hoc multiple comparisons. All biochemical analyses (ELISA, densitometry, RT-PCR data) were analyzed using Student's t test or one-way ANOVA, followed by Tukey's post hoc test. Statistically significant group differences were set at $P < 0.05$.

Results

Effect of peony treatment on repeating injection of CORT-induced behavior characterization

Mouse depression model was established via repeated subcutaneous injection of CORT for 21 consecutive days with concentration of 20 mg/kg/day. After repeated injections, CORT concentration in the serum of depressant mouse was analyzed using a ELISA kit. The open field test and tail suspension test were

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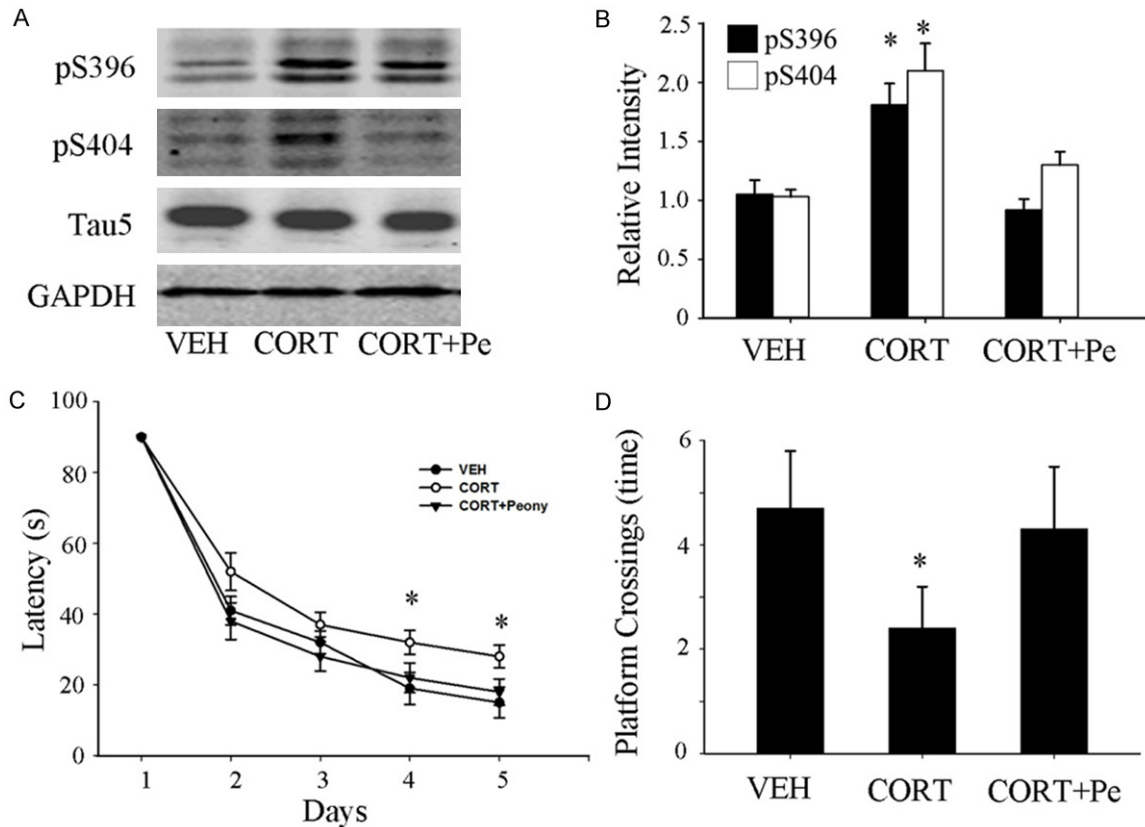


Figure 2. (A) Effects of peony on hyperphosphorylation level of tau in mouse depression model and its volume graphics (B) After treating with peony, depressant mice also test in morris water maze for 5 consecutive day, their time spent in finding platform (C) and times cross the platform (D) were record. * $P < 0.05$, vs VEH. VEH, vehicle; CORT+Pe, 20 mg/kg subcutaneous injection of corticosterone and gavage treated of peony at dose of 80 mg/kg.

used to test the anxiety/depression-like level of the mice. As shown in **Figure 1A**, CORT levels was enhanced significantly after 3 weeks' injection in CORT group as compared to VEH group (55.3 ± 5.6 ng/ml vs 105.5 ± 10.5 ng/ml, $P < 0.05$), and decreased almost to normal following peony treatment (68.5 ± 8.6 ng/ml). Depression-like behavior of mice was assessed using open field test and tail suspension test. As shown in **Figure 1B**, time spent in the center zone of the box decreased significantly in CORT group (146.4 ± 19.4 s vs VEH 220.4 ± 24.2 s, $P < 0.05$), and peony administration could partly improve this performance (205 ± 16.5 s). Chronic exogenous corticosterone group or peony treatment group had no impact on total distance recorded among three groups in open field test (**Figure 1D**, VEH 3105 ± 240 cm vs CORT 3215 ± 410 cm vs CORT+Pe 3310 ± 290 cm, $P > 0.05$). Mobility time of mice treated daily with CORT decreased significantly (175 ± 17 s vs VEH 242 ± 24 s, $P < 0.05$), and was reversed after peony

treatment as shown in tail suspension test in **Figure 1C** (234 ± 14 s). Our results revealed that mice exerted depression-like behavior after CORT injection for 3 weeks, and peony could ameliorate CORT-induced deficient behavior.

Peony treatment reverses mouse memory deficiency, and decreases tau hyperphosphorylation level

Chronic stress has been reported to induce hyperphosphorylation of tau in multiple sites in the hippocampus of animals. Hyperphosphorylation of tau and impairment of spatial memory are typical symptom of AD in the early stages. To investigate whether peony could ameliorate 3 weeks' injection of CORT induced tau-hyperphosphorylation, we quantified the phosphor-tau using western blot. Protein analysis of mice's hippocampus shown that, tau hyperphosphorylation level at site Ser404 and Ser396 were enhanced significantly with treatment of

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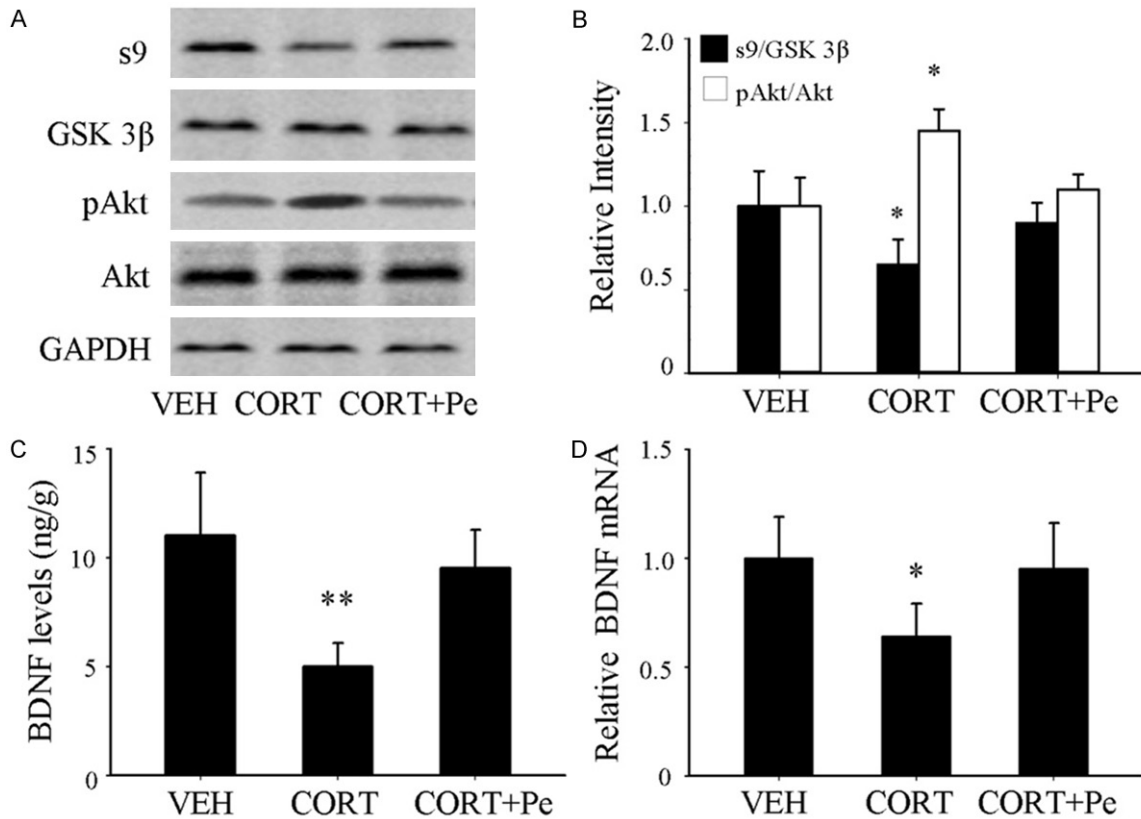


Figure 3. Signaling pathway was altered after treatment with peony in depressant mice. GSK 3 β phosphorylation level in s9 site (inactive form) and total GSK 3 β , Akt phosphorylation level (pAkt) in s473 site and total Akt were examined using western blot (A) The values of s9 was normalized against the amount of GSK 3 β and GAPDH, and pAkt was normalized against the amount of Akt and GAPDH (B, C) BDNF levels increase in the hippocampus of peony-treated mouse as detected by ELISA. (D) Real time PCR of RNA extract of the hippocampus mouse in three groups revealed comparable levels of BDNF mRNA. (* $P < 0.05$ for CORT vs VEH; ** $P < 0.01$ for CORT vs VEH).

CORT ($P < 0.05$), and reversed following peony treated (Figure 2A and 2B). Furthermore, mice's deficient in learning and memory was tested in Morris water maze. Compared to control group (VEH), CORT significantly prolonged the latency of mice to find the hidden platform on day 4 and 5 (Figure 2C), and times cross the platform was also decreased (2.4 ± 0.8 vs VEH 4.7 ± 1.1 , $P < 0.05$) (Figure 2D). Supplementation of peony partially restored the CORT-induced impairment in learning memory (Figure 2D).

Effect of peony on signaling pathway of repeating injection of corticosterone in mouse hippocampus

Previous reports had shown that brain-derived neurotrophic factor (BDNF) stimulated TrkB, and then activates various signal transduction cascades, including PI3K/AKT, PKC and PKA pathways. These pathways has been proven to

affect tau phosphorylation levels independently [17]. GSK 3 β is one of the major downstream substrates of Akt in the PI3K/Akt-dependent signaling pathway. Thus, we tested the expression levels of phosphorylated Akt and GSK 3 β using antibodies that recognize phosphorylation of Akt at Serine 473 (pAkt 473) and of GSK 3 β at Serine 9 (pGSK 3 β s9) in the hippocampus of mice. We observed a significant decrease of pGSK 3 β s9 and increase of pAkt 473 in 3-weeks' injection of corticosterone, and they were reversed after treating with peony (Figure 3A and 3B). Furthermore, We observed a decrease in BDNF expression level in the hippocampus of CORT mice using ELISA assay compared to vehicle treated littermates mice (Figure 3C). Following peony treatment, BDNF's level had a two fold increase compared to CORT treated group, though less than normal group (Figure 3C). To gain more insight, we sought to examine whether peony induced upregulation

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of BDNF mRNA. Here we show that BDNF mRNA level was significantly upregulated following peony in mice (**Figure 3D**).

Discussion

Peony is a traditional Chinese herbal formulae for the treatment of depression-like symptoms via inhibiting activity of HPA axis [18]. It has been reported that a glycoside-rich extract of peony could reverse the reduction of BDNF induced by chronic unpredictable mild stress in rats [18]. In present study, we have demonstrated that peony can attenuate hyperphosphorylation level of tau and learning and spatial memory deficient induced by repeating corticosterone injection in mice. PI3K-Akt-GSK 3 β pathway was suggested involve in tau hyperphosphorylation induced by corticosterone. Furthermore, our results indicated that BDNF may participate in this process, whose protein level was declined after treatment with corticosterone and reversed by peony.

Elevated cortisol had a closed link with depressive symptomatology. Numbers of studies reported that patients experiencing elevated glucocorticoid levels as a result of synthetic glucocorticoid therapy or Cushing's disease develop psychiatric and cognitive symptoms consistent with those observed in major depression [19, 20]. Clinical observations had shown elevated cortisol levels in Alzheimer patients [21]. Based on these clinical findings, many animal models were developed to further study the underlying neurobiological mechanisms of depression. It's suggested that repeated corticosterone injections can reliably prolong immobility and increase depression-like behavior in a dose-dependent manner in mice [22], thus we applied such animal model in this study. Consistent with previous reports, 21 consecutive days repeating injection of corticosterone induced depression-like behavior in mice. Though some reported that body weight may loss after corticosterone injection, we didn't observe significant difference in mice's body weight in this study. As expected, mice's capacity in learning and memory, tested by morris water maze, was also impaired after 3 weeks' injection of corticosterone. Tau's hyperphosphorylation at several site (pS396 and pS404) also enhanced after corticosterone induction. Altogether, 3 weeks' induction by corticosterone could make mice exert depression-like and dementia-like behavior.

Tau phosphorylation has a key role in development and progress of AD and dementia [23]. Several protein kinases may take part in these pathological processes, including cyclin-dependent kinase 5 (cdk-5), GSK-3, and protein kinase A (PKA). GSK-3 regulates tau hyperphosphorylation at ser198/ser199/ser202 sites and ser396/ser404 sites [24, 25]. In depression-stress-anxiety phenotypes, GSK-3 β is disinhibited by the attenuation of survival pathway BDNF-PI3K/Akt that inhibits it. GSK-3 β inhibitors lithium and other agents display anti-manic and antidepressant effects in some animal models [26]. In present study, ser9-GSK 3 β (inactive form of GSK 3 β) protein level was observed declined, consistent with other depression animal model [27, 28]. These results indicated GSK 3 β owned a key role in both development of depression and AD. It's a potential target for pharmaceutical therapy.

BDNF, one of the major neurotrophic factors, has key roles in the maintenance and survival of neurons, synaptic integrity, and synaptic plasticity [29]. BDNF may mitigate the deleterious effects of AD pathology in the brains of older adults, and low serum or plasma BDNF levels may predict more rapid cognitive decline and may be lower in AD [30-32]. Hippocampus of some dementia animal models shows an age-dependent decrease in BDNF, which is correlated with memory loss and a decrease of memory [33]. The first study showing the role of BDNF in stress was that immobilization stress in rats lowers the expression of BDNF in the hippocampus [34]. Other stressors, such as social defeat, were also reported to have decrease in BDNF in hippocampus [35]. Recently, it's reported that corticosterone treatment in mice reduces BDNF expression in the hippocampus [36]. In this study, we observed obviously declined protein and mRNA level of BDNF in CORT group compared with control group after 3 weeks' induction of corticosterone, and peony could partl restore CORT-induced decreased of BDNF. Altogether, our results suggests that BDNF may be involved in attenuating CORT-induced tau hyperphosphorylation and spatial memory deficit.

In summary, we have found that peony can prevent CORT-induced overactivation of GSK 3 β and thus lead to spatial memory impairment and tau hyperphosphorylation. And peony maybe involved in this process.

Disclosure of conflict of interest

None.

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References

[1] Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J Alzheimers Dis* 2001; 3: 75.

[2] Wang JZ, Liu F. Microtubule-associated protein tau in development, degeneration and protection of neurons. *Prog Neurobiol* 2008; 85: 148.

[3] Vyas S, Maatouk L. Contribution of glucocorticoids and glucocorticoid receptors to the regulation of neurodegenerative processes. *CNS Neurol Disord Drug Targets* 2013; 12: 1175.

[4] Wang JZ, Gao X, Wang ZH. The physiology and pathology of microtubule-associated protein tau. *Essays Biochem* 2014; 56: 111.

[5] Wang JZ, Xia YY, Grundke-Iqbal I, Iqbal K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. *J Alzheimers Dis* 2013; 33 Suppl 1: S123.

[6] Briones TL, Darwish H. Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden. *J Neuroinflammation* 2012; 9: 244.

[7] Zhang LF, Shi L, Liu H, Meng FT, Liu YJ, Wu HM, Du X, Zhou JN. Increased hippocampal tau phosphorylation and axonal mitochondrial transport in a mouse model of chronic stress. *Int J Neuropsychopharmacol* 2012; 15: 337.

[8] Briones A, Gagno S, Martisova E, Dobarro M, Aisa B, Solas M, Tordera R, Ramirez M. Stress-induced anhedonia is associated with an increase in Alzheimer's disease-related markers. *Br J Pharmacol* 2012; 165: 897.

[9] Hernandez F, Lucas JJ, Avila J. GSK3 and tau: two convergence points in Alzheimer's disease. *J Alzheimers Dis* 2013; 33 Suppl 1: S141.

[10] Maqbool M, Mobashir M, Hoda N. Pivotal role of glycogen synthase kinase-3: A therapeutic target for Alzheimer's disease. *Eur J Med Chem* 2016; 107: 63.

[11] Ren QG, Gong WG, Wang YJ, Zhou QD, Zhang ZJ. Citalopram attenuates tau hyperphosphorylation and spatial memory deficit induced by social isolation rearing in middle-aged rats. *J Mol Neurosci* 2015; 56: 145.

[12] Wang R, Xu Y, Wu HL, Li YB, Li YH, Guo JB, Li XJ. The antidepressant effects of curcumin in the forced swimming test involve 5-HT1 and 5-HT2 receptors. *Eur J Pharmacol* 2008; 578: 43.

[13] Zhou H, Zhang AP, Chen MZ. The modulatory effects of TGP on hypothalamo-pituitary-adrenal axis and immune function in stress rats. *Chinese Pharmacological Bulletin* 1994.

[14] Mao QQ, Xian YF, Ip SP, Tsai SH, Che CT. Protective effects of peony glycosides against corticosterone-induced cell death in PC12 cells through antioxidant action. *J Ethnopharmacol* 2011; 133: 1121.

[15] Ma Z, Ma Y, Xia Q, Li Y, Li R, Chang W, Chen J, Leng Z, Tao K. MicroRNA-155 expression inversely correlates with pathologic stage of gastric cancer and it inhibits gastric cancer cell growth by targeting cyclin D1. *J Cancer Res Clin Oncol* 2016; 142: 1201.

[16] Szapacs ME, Mathews TA, Tessarollo L, Ernest LW, Mamounas LA, Andrews AM. Exploring the relationship between serotonin and brain-derived neurotrophic factor: analysis of BDNF protein and extraneuronal 5-HT in mice with reduced serotonin transporter or BDNF expression. *J Neurosci Methods* 2004; 140: 81.

[17] Hu YS, Long N, Pigino G, Brady ST, Lazarov O. Molecular mechanisms of environmental enrichment: impairments in Akt/GSK3beta, neurotrophin-3 and CREB signaling. *PLoS One* 2013; 8: e64460.

[18] Mao QQ, Ip SP, Xian YF, Hu Z, Che CT. Anti-depressant-like effect of peony: a mini-review. *Pharm Biol* 2012; 50: 72.

[19] Brown ES, J Woolston D, Frol A, Bobadilla L, Khan DA, Hanczyc M, Rush AJ, Fleckenstein J, Babcock E, Cullum CM. Hippocampal volume, spectroscopy, cognition, and mood in patients receiving corticosteroid therapy. *Biol Psychiatry* 2004; 55: 538-45.

[20] Antonijevic IA, Steiger A. Depression-like changes of the sleep-EEG during high dose corticosteroid treatment in patients with multiple sclerosis. *Psychoneuroendocrinol* 2003; 28: 780.

[21] Mondadori C, Gentsch C, Hengerer B, Ducret T, Borkowski J, Racine A, Lederer R, Haeusler A. Pretreatment with aldosterone or corticosterone blocks the memory-enhancing effects of nimodipine, captopril, CGP 37,849, and strychnine in mice. *Psychopharmacology (Berl)* 1992; 109: 383.

[22] Zhao Y, Ma R, Shen J, Su H, Xing D, Du L. A mouse model of depression induced by repeated corticosterone injections. *Eur J Pharmacol* 2008; 581: 113.

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- [23] Liu SJ, Zhang JY, Li HL, Fang ZY, Wang Q, Deng HM, Gong CX, Grundke-Iqbal I, Iqbal K, Wang JZ. Tau becomes a more favorable substrate for GSK-3 when it is prephosphorylated by PKA in rat brain. *J Biol Chem* 2004; 279: 50078.
- [24] Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A* 1986; 83: 4913.
- [25] Qu ZS, Li L, Sun XJ, Zhao YW, Zhang J, Geng Z, Fu JL, Ren QG. Glycogen synthase kinase-3 regulates production of amyloid-beta peptides and tau phosphorylation in diabetic rat brain. *ScientificWorldJournal* 2014; 2014: 878123.
- [26] Du J, Wei Y, Liu L, Wang Y, Khairova R, Blumenthal R, Tragon T, Hunsberger JG, Machado-Vieira R, Drevets W, Wang YT, Manji HK. A kinesin signaling complex mediates the ability of GSK-3beta to affect mood-associated behaviors. *Proc Natl Acad Sci U S A* 2010; 107: 11573.
- [27] Ren QG, Gong WG, Wang YJ, Zhou QD, Zhang ZJ. Citalopram attenuates tau hyperphosphorylation and spatial memory deficit induced by social isolation rearing in middle-aged rats. *J Mol Neurosci* 2015; 56: 145.
- [28] Yang C, Guo X, Wang GH, Wang HL, Liu ZC, Liu H, Zhu ZX, Li Y. Changes in tau phosphorylation levels in the hippocampus and frontal cortex following chronic stress. *Braz J Med Biol Res* 2014; 47: 237.
- [29] Dwivedi Y. Involvement of brain-derived neurotrophic factor in late-life depression. *Am J Geriatr Psychiatry* 2013; 21: 433.
- [30] Laske C, Stellos K, Hoffmann N, Stransky E, Straten G, Eschweiler GW, Leyhe T. Higher BDNF serum levels predict slower cognitive decline in Alzheimer's disease patients. *Int J Neuropsychopharmacol* 2011; 14: 399.
- [31] Boiocchi C, Maggioli E, Zorzetto M, Sinforiani E, Cereda C, Ricevuti G, Cuccia M. Brain-derived neurotrophic factor gene variants and Alzheimer disease: an association study in an Alzheimer disease Italian population. *Rejuvenation Res* 2013; 16: 57.
- [32] Voineskos AN, Lerch JP, Felsky D, Shaikh S, Rajji TK, Miranda D, Lobaugh NJ, Mulsant BH, Pollock BG, Kennedy JL. The brain-derived neurotrophic factor Val66Met polymorphism and prediction of neural risk for Alzheimer disease. *Arch Gen Psychiatry* 2011; 68: 198.
- [33] Hwang IK, Yoo KY, Jung BK, Cho JH, Kim DH, Kang TC, Kwon YG, Kim YS, Won MH. Correlations between neuronal loss, decrease of memory, and decrease expression of brain-derived neurotrophic factor in the gerbil hippocampus during normal aging. *Exp Neurol* 2006; 201: 75.
- [34] Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 1995; 15: 1768.
- [35] Pizarro JM, Lumley LA, Medina W, Robison CL, Chang WE, Alagappan A, Bah MJ, Dawood MY, Shah JD, Mark B, Kendall N, Smith MA, Saviolakis GA, Meyerhoff JL. Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain Res* 2004; 1025: 10.
- [36] Mao QQ, Ip SP, Ko KM, Tsai SH, Zhao M, Che CT. Peony glycosides protect against corticosterone-induced neurotoxicity in PC12 cells. *Cell Mol Neurobiol* 2009; 29: 643.