Antidepressant Effects of a Plant-Derived Flavonoid Baicalein Involving Extracellular Signal-Regulated Kinases Cascade

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Depression and related mood disorders are among the world's greatest public health problems. Previous studies have demonstrated that baicalein (Bai), one plant-derived active flavonoid, exhibits neuroprotection against ischemic brain injury and stimulates the levels of phosphorylation of extracellular signal-regulated kinase (pERK) and brain-derived neurotrophic factor (BDNF) expression *in vivo*. In this study, the antidepressant-like effects of baicalein was investigated using acute and chronic animal models of depression. The results showed that acute application of Bai at doses of 1, 2 and 4 mg/kg by intraperitoneal injection (i.p.) significantly reduced the immobility time in the forced swimming test (FST) and tail suspending test (TST) of mice. In addition, the chronic application of Bai by i.p. for 21 d also reduced the immobility time and improved locomotor activity in chronic unpredictable mild stress (CMS) model rats. Furthermore, it was shown that Bai reversed the reduction of extracellular ERKs phosphorylation and the level of BDNF expression in the hippocampus of CMS model rats. These results suggest that Bai produce an antidepressant-like effect and this effect is at least partly mediated by hippocampal ERK-mediated neurotrophic action.

Key words chronic unpredictable mild stress; baicalein; forced swimming test; tail suspending test

Depression is a serious psychiatric disorder that easily causes physical impairment and a high suicide rate.[1)] Current treatment mainly depends on the traditional therapeutic strategy that affecting serotonin and/or norepinephrine system, but roughly half of affected individuals are inadequately treated by available medications and psychotherapeutic approaches.[2,3)] Despite tremendous effort, the field has not yet succeeded in developing fundamentally new antidepressants with distinct mechanisms of action.[4)]

It has been demonstrated that adult neurogenesis in the dentate gyrus (DG) plays a critical role in depression and the therapeutic effects of antidepressants appear to be neurogenesis-dependent.[5)] Neurotrophic factors such as brain-derived neurotrophic factor (BDNF), which act as critical regulator of memory and synaptic plasticity, produce the up-regulation of neurogenesis.[6)] It is well known that BDNF has been shown to enhance neurogenesis. In addition, BDNF initiates TrkB receptor-dependent activation of extracellular signal-regulated kinases (ERKs) and exhibits beneficial effect for the treatment of depression.[7)] ERKs is the well-studied member of the mitogen-activated protein kinase (MAPK) family and ERK pathway is the major convergence point involved in neurotrophic and other signal pathways that regulates cellular differentiation and neuronal plasticity.[8—10)] It has been demonstrated that ERK activation can further trigger neurotrophic effects, including neurite growth, neuronal survival and hippocampal neurogenesis.[11)] Thus, much attention has turned to the role of ERKs in depression. Actually, many classic antidepressant drugs, such as fluoxetine, can reverse the decreased ERK1/2 phosphorylation (p-ERK1/2) induced by depression in the hippocampus.[12)] By the fact that the therapeutic effects of antidepressants appear to be ERK-related, we postulate that agents that can reverse the decrease in BDNF and p-ERK1/2 caused by depression may produce antidepressant effects.

Accumulated evidence has shown that plant-derived flavonoids can produce wide effects including neuroprotection, antioxidant, and regulation of synaptic plasticity and memory enhancement.[13—16)] Recently, it is demonstrated that some flavonoids such as hypericum perforatum and quercetin, display antidepressant effects and attenuate the cognitive deficits caused by major depression.[17—19)] Baicalein (Bai) is one of the most active flavonoids found in the dry roots of Scutellaria baicalensis Georgi. It is documented that Bai can get across blood–brain barrier (BBB).[13,20)] In our previous study, it is shown that Bai protects neurons from free radical-induced damage *in vitro* and alleviates ischemic brain injury *in vivo*.[13,21)] More importantly, several reports have revealed that Bai stimulates the expression levels of pERK and BDNF in the hippocampus of normal rats.[22)] Considering this neurotrophic effect, we postulate that Bai may have an antidepressant-like effect. Thus, the therapeutic role of Bai in animals of depression and its potential mechanisms were investigated through behavioral tests and Western blot analysis.

MATERIALS AND METHODS

Animals and Drug Administration Adult male Kunming (KM) mice (18—22 g) and Sprague-Dawley (SD) rats (160—180 g) were obtained from the Experimental Animals Center of Tongji Medical College, Huazhong University of Science and Technology. All animals were housed in groups under standard conditions (12 h light–dark cycle; light on 7 a.m.—7 p.m.; temperature of 22±1 °C) with free access to water and food, and allowed to acclimate a week. The experimental protocols were approved by the Committee of Animal

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Care of Huazhong University of Science and Technology. Bai (molecular weight: 270.24, purity above 98.0%, Fig. 1) was purchased from Sigma (Product No. 11712). Bai was dissolved in saline and the pH value was adjusted 10 with NaOH as described in our previous publication.[18,19]

In the experiments of acute treatment, the mice were subjected to the open field test (OFT), the forced swimming test (FST) and tail suspending test (TST), respectively (Fig. 2A). In each test, the mice were randomly divided into five groups ($n=10$ per group), *i.e.*, control group, 15 mg/kg imipramine (IMI) group, 1 mg/kg, 2 mg/kg and 4 mg/kg Bai (Bai) group. The mice in control group were administrated intraperitoneal injection (i.p.) of equal volume of vehicle (0.2% NaOH). The mice at 1 mg/kg, 2 mg/kg and 4 mg/kg in Bai group were given by i.p., respectively. The mice in IMI group were given by i.p. administration of 15 mg/kg IMI. All the animals were administered their respective treatment 1 h prior to the TST and FST. In OFT, the animals were measured locmotor activity 24 h after administration.

In the experiments of chronic treatment, two different protocols were performed (Fig. 2B). One protocol was administrated with Bai for 7 d to explore the potential chronic antidepressant action and behavior changes of Bai in non-stressed rats. The rats were randomly divided into five groups ($n=10$ per group), *i.e.*, control group, 15 mg/kg IMI group, 1 mg/kg, 2 mg/kg and 4 mg/kg Bai group for FST and OFT, respectively. The control rats were administrated (i.p.) equal-volume of vehicle (0.2% NaOH). The rats in Bai group were given an i.p. injection of 1, 2 and 4 mg/kg Bai, respectively. The rats in IMI group were given an i.p. injection of 15 mg/kg IMI. All the animals were administered their respective treatment once daily for 7 d. The last administration was conducted 1 h before the FST and OFT. The other protocol was administrated with Bai for 21 d following by chronic unpredictable mild stress (CMS) to investigate the antidepressant-like property in animal model of the depression. However, no stressor was performed to the animals on 8th and 15th day in order to conduct their OFT. The rats were randomly divided into six groups ($n=10$ per group), *i.e.*, control group, CMS group, 15 mg/kg IMI group, 1 mg/kg, 2 mg/kg, 4 mg/kg Bai group. The control rats were administrated (i.p.) equal-volume of vehicle (0.2% NaOH). The rats in Bai group were given an i.p. injection of 1, 2 and 4 mg/kg Bai, respectively. The rats in IMI group were applied by intraperitoneal injection of 15 mg/kg IMI. All the animals were administered their respective treatment once daily for 21 d. On 23rd day, the rats were tested in open field test and FST.

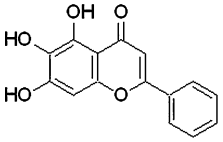

Fig. 1. The Chemical Structure of Bai

TST and FST in Mice TST and FST were described by Steru *et al.*, and Lucki.[23,24] Each mouse was individually suspended by the tail from the edge of a shelf 70 cm above the floor. Total duration of immobility during the 6-min test was recorded. When each mouse hung motionlessly and passively, the behavior was considered as immobility. In FST, mice were placed individually in a vertical glass cylinder (height: 30 cm; diameter: 10 cm) containing 10 cm of water maintained at 22 ± 1 °C. The duration of immobility was measured only during the last 4-min of the total 6-min test period. Immobility was defined as floating motionless or only making those movements necessary to keep the nose above the water.

FST in CMS Rat The chronic stress procedure was adopted from a previous study with a little modification.[25] Table 1 was the procedure of various unpredictable mild stress. These stressors were randomly applied at once daily and lasted for 21 d. The FST in rats were measured on 23rd

Table 1. The Procedure of CMS

Number	Type of stressor
1	1-h shaking crowding, 240 Hz with 6 rats in a box
2	the food deprivated for 24 h
3	15-min cold swimming (17 °C)
4	10-min tail pinch (a clothespin placed at 1 cm from the base of tail)
5	the water deprivated for 24 h
6	10-min inescapable shock (1.5 mA, 15 s on, interval 60 s)
7	15-min warm swimming (37 °C)
8	30-min restraint with each rat in a bottle
9	24-h high density of housing (6 rats per cage)
10	24-h separation

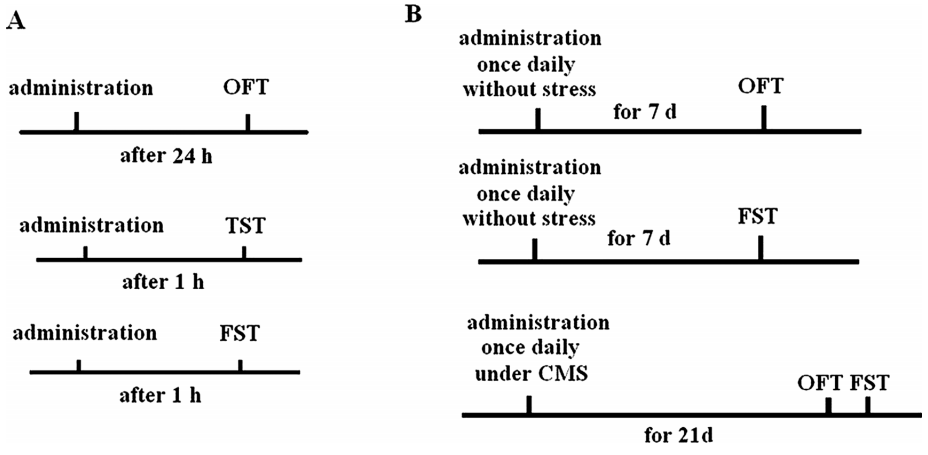

Fig. 2. The Administrative Procedure in Experimental Situations

(A) The administrative procedure in acute treatment. (B) The administrative procedure in chronic treatment.

day according to the traditional method described by Porsolt *et al.*[26] Before testing, the rats were placed individually in clear glass cylinders (40 cm height×18 cm diameter) filled with water (21—23 °C) to a depth of 20 cm for 15 min. In the test session, the rats were exposed to the cylinders that were the same as FST in mice. The duration of immobility was measured only during the last 4-min of the total 6-min test period.

Open Field Test (OFT) Decreased locomotor activity has been used as an index of low emotionality in rats and to evaluate the degree of depression.[27] In this study, OFT was used to detect locomotor activity in each rat according to the method used by Ferreira *et al.*[28] No stressor was performed to the animals for at least 24 h before OFT. The open field was made of black wood (100×100×25 cm), which was divided into 25 (5 cm×5 cm) identical sectors by white stripes. The field was further divided into central and peripheral sector, where the central sector contained the 9 central squares (3 cm×3 cm) and the peripheral sector were the remaining squares.[27] Locomotor activity was measured in non-stressed mice at 24 h after administration of Bai. After administration for 7 d, the locomotor activity of rat was measured on 8th day without stress. In CMS experiment, the rat was placed individually in the center of open-field apparatus, the locomotor activity was measured on the 9th, 16th and 23th day. Locomotion (number of line crossing within 5 min) and rearing frequencies (the number of times an animal stood on its hind legs) were adopted to evaluate the locomotor activity.

Western Blotting The rats were randomly selected and sacrificed by decapitation at the end of FST in CMS, the brains were rapidly removed on ice and dissected into cerebral slice by slicing machine. Then cerebral slices were isolated for DG of hippocampus under a microscope. It was isolated and similar with previous studies from our laboratory,[29] hippocampal homogenates were lysed on ice for 30 min in lyses buffer (50 mM Tris–HCl, pH 7.4, 1 mM ethylenediamine tetraacetic acid (EDTA), 100 mM NaCl, 20 mM NaF, 3 mM Na_3VO_4, 1 mM phenylmethyl sulfonylfluoride (PMSF), with 1% Nonidet P-40, and protease inhibitor cocktail). The lysates were centrifuged at 12000×***g*** for 15 min, and the supernatant was recovered. Equal amount protein samples were separated by 10% sodium dodecylsulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and then transferred to nitrocellulose membranes. Transferred membranes were incubated overnight at 4 °C with different primary antibodies (ERK1/2 1 : 1000 dilution, Abcam; p-ERK1/2 1 : 500 dilution, Santa Cruz; BDNF 1 : 500 dilution, Santa Cruz). On the second day, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1 : 5000) for 1 h at room temperature. After repeated washes, membranes were reacted with enhanced chemiluminescence reagents (Super-Signal West Pico; Pierce) and visualized with X-ray films (Kodak X-Omat, Rochester, NY, U.S.A.). The films were scanned and the optical density of the bands was determined using Optiquant software (Packard Instruments, the Netherlands).

Data Analysis The data were expressed as mean±S.E.M. Behavioral and biochemical data were analyzed using a one-way analysis of variance (ANOVA). Following ANOVA analyses, Dunnett's *post hoc* tests were used. Analyses were performed using the software SPSS 11.5 for windows. Statistical significance was accepted at the conventional $p<0.05$ level.

RESULTS

Acute Treatment of Bai Decreases the Immobility Time in TST and FST of Mice without Effect on Locomotor Activity in OFT To explore preliminary antidepressant action by Bai in CNS, the acute administration with Bai was performed. Bai at the doses of 1, 2 and 4 mg/kg and 15 mg/kg IMI had no influence on locomotor activity compared with control group in OFT ($n=10$, Figs. 3A, B), suggesting that Bai did not produce intensive excitatory effect and behavioral damage in CNS under non-stress conditions of mice. However, Bai at the doses of 1, 2 and 4 mg/kg reduced the duration of immobility in the TST in a dose-dependent manner. The immobility time was reduced to 50.10±10.17 s, 45.60±12.97 s and 37.30±13.69 s compared with the control group, respectively ($p<0.05$ *vs.* control group, $n=10$, Fig. 3C). In the FST, the same doses of Bai also significantly decreased immobility time to 53.44±9.56 s, 56.00±14.81 s and 50.56±11.65 s, respectively ($p<0.05$ *vs.* control group, $n=10$, Fig. 3D), respectively. Meanwhile, the effects of Bai were similar to the classical antidepressant IMI. In both behavioral models of depression, the immobility time was significantly decreased by IMI to 32.20±10.55 s and 57.00±12.4 s ($p<0.05$ *vs.* control group, $n=10$, Figs. 3C, D), respectively. These data indicated that Bai decreased the immobility time in TST and FST in mice similar to the IMI, suggesting that Bai might have an antidepressant effect.

Chronic Treatment of Bai Reduces the Immobility Time in FST of Rats By the fact that the effect of antidepressant often appears after a few weeks in clinic, we investigated the effects of chronic Bai treatment, in which Bai was administrated once daily for 7 d but no underwent CMS in rats. As shown in Fig. 4A, treatments with Bai at 1, 2, 4 mg/kg and IMI at 15 mg/kg, the immobility time was significantly reduced compared with control group (36.8±4.02 s), and the values were 31.7±6.58 s, 33.1±3.46 s, 28.6±7.72 s and 28.56±5.14 s, respectively ($p<0.05$ *vs.* control group, $n=10$, Fig. 4A). To further investigate the chronic antidepressant effects of Bai, the rats were underwent CMS for 21 d. As shown in Fig. 4B, after 21 d CMS, the immobility time of CMS rats in FST was obviously increased to 130.30±25.37 s ($p<0.05$ *vs.* control group, $n=10$). However, Bai at 1, 2 and 4 mg/kg effectively reduced the immobility time to 102.10±20.86 s, 87.89±18.94 s and 81.11±17.77 s ($p<0.05$ *vs.* model group, $n=10$), respectively, which were similar to the effect of the classic antidepressant IMI (15 mg/kg, i.p.), and the immobility time in the latter group was decreased to 78.14±22.78 s ($p<0.05$ *vs.* model group, $n=10$). These results showed that chronic Bai treatment also improved the immobility time in the depression of rats.

Effects of Bai on Locomotor Activity in Open-Field Test To explore the excitatory effect of Bai on the behavior CNS, the locomotor activity of rats under non-stress conditions was measured. As shown in Fig. 5A, after 7 d, the number of line crossing was 51.52±5.63 times in control group. After administration with Bai and IMI once daily for 7 d, their number of line crossing were 43.21±3.13, 45.82±3.12, 47.59±2.10 and 49.51±3.13 times, respectively ($n=10$, Fig.

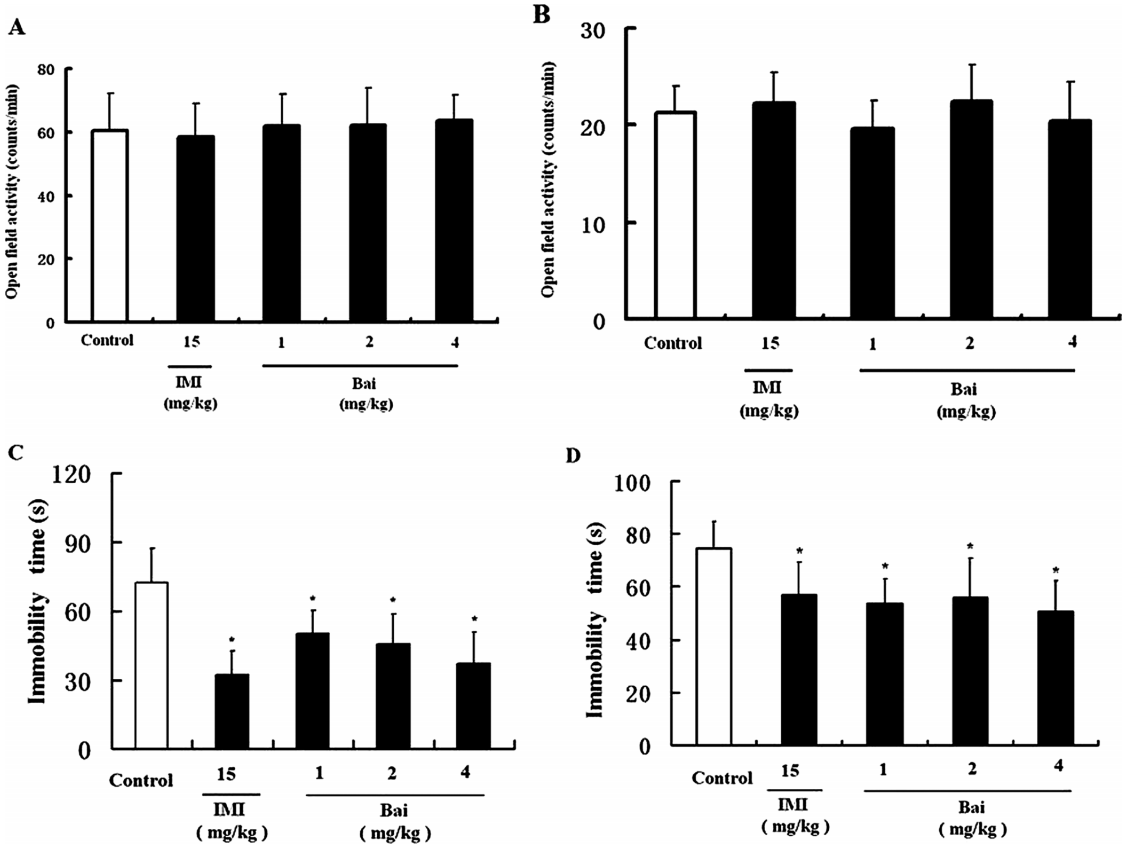

Fig. 3. Acute Treatment of Bai Decreases the Immobility Time in TST and FST of Mice without Effect on Locomotor Activity in OFT Test

(A) The number of line crossing in the OFT in non-stressed mice. (B) The rearing frequencies in the OFT in non-stressed mice. (C) Immobility time in the TST. (D) Immobility time in the FST. Control (vehicle), IMI (15 mg/kg) and Bai (1, 2, 4 mg/kg) were administrated (i.p.) 1 h prior to the test, respectively. Each column and bar represented means±S.E.M. (n=10). $*p<0.05$ *vs.* control.

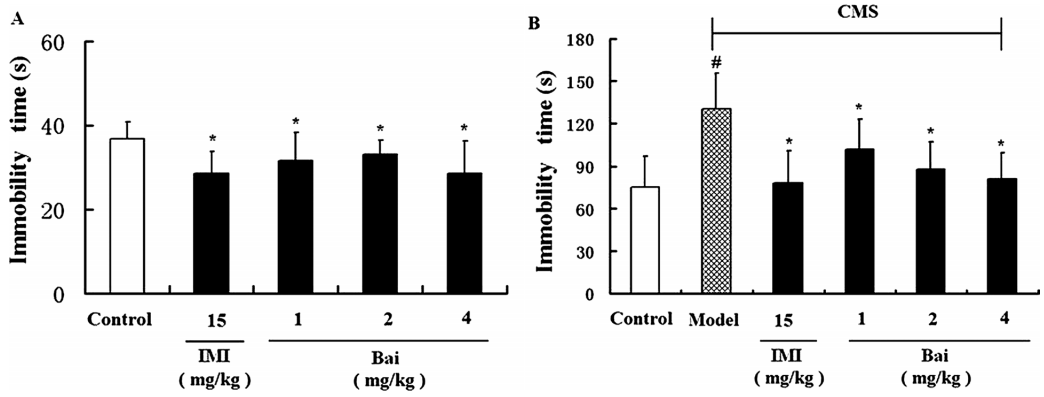

Fig. 4. Bai Reduces the Immobility Time in FST of Rats

(A) The immobility time in FST of normal rats. Control (vehicle), IMI (15 mg/kg) and Bai (1, 2, 4 mg/kg) were administrated (i.p.) for 7 d, respectively. (B) The immobility time in FST for CMS model. Control (vehicle), IMI (15 mg/kg) and Bai (1, 2, 4 mg/kg) were administrated (i.p.) for 21 d, respectively. Each column and bar represented as means±S.E.M. (n=10). $\#p<0.05$ *vs.* control group, $*p<0.05$ *vs.* model group.

5A). Another index, rearing frequencies (number of times that the animal stood on its hind legs), which was also reflected locomotion activity was 15.31±2.12 times in control group in Fig. 5B. Compared with control group, rearing frequencies in Bai and IMI group were not significantly different (n=10, Fig. 5B), indicating that Bai does not produce excitatory effect on behavior in CNS under non-stress conditions. The CMS stressed rats showed an evident change in locomotor activity in the open field test. When the rats were placed in a novel environment, a decrease in spontaneous activity in the CMS mice was observed. In 0 week, there were no significant differences between the every group. After 21 d, the number of line crossing was reduced from 45.60± 6.28 to 16.00±2.47 times in the CMS rats ($p<0.05$ *vs.* control group, n=10, Fig. 5C). Treatment with Bai (4 mg/kg) and IMI (15 mg/kg) caused a gradual recovery of the number of line crossing and the values were 34.60±5.58 and 32.3±4.53 times, respectively ($p<0.05$ *vs.* model group, n=10, Fig. 5C). Rearing frequencies were decreased from 12.00±1.20 to 4.90±1.59 times in CMS rats ($p<0.05$ *vs.*

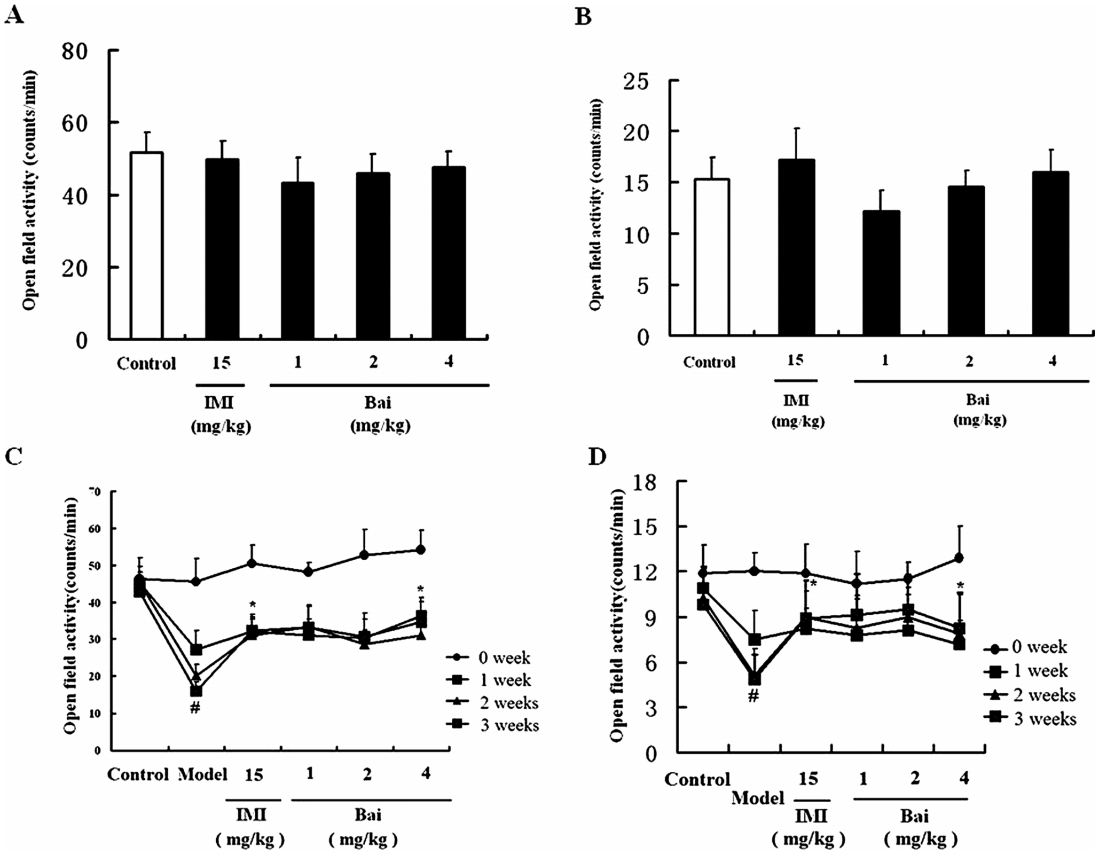

Fig. 5. Effects of Bai on Locomotor Activity in Open Field Test

(A) The number of line crossing in control, model and drug administration groups within 7 d without stress. (B) The rearing frequencies in control, model and drug administration groups within 7 d under without stress. (C) The number of line crossing in control, model and drug administration groups within 21 d under CMS. (D) The rearing frequencies in control, model and drug administration groups within 21 d under CMS. Control (vehicle), IMI (15 mg/kg) and Bai (1, 2, 4 mg/kg) were administrated (i.p.) for 7 d or 21 d respectively. Each line represented as means±S.E.M. (n=10). $^{\#}p<0.05$ *vs.* control group, $^{*}p<0.05$ *vs.* model group.

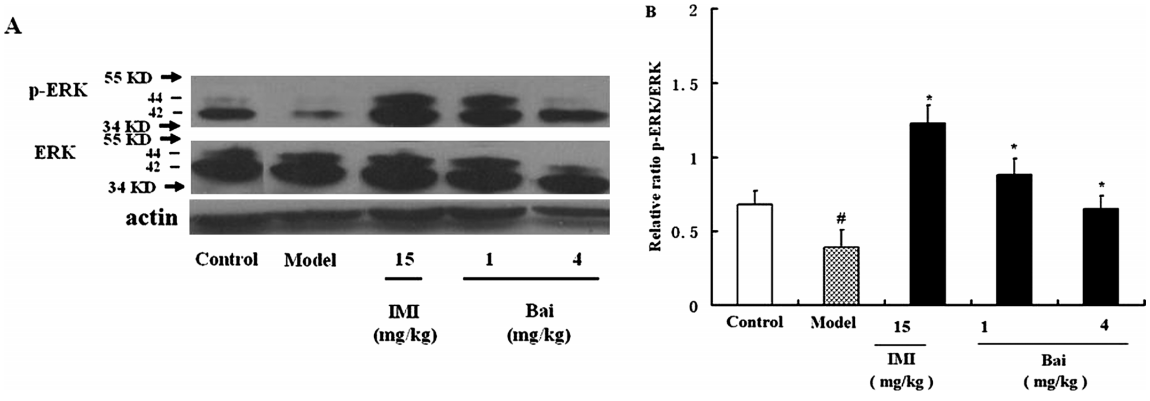

Fig. 6. Bai Increases the Expression of Phosphorylation of ERK1/2 in the Hippocampus of CMS Rats

(A) Western blot analysis indicates the changes of p-ERK1/2, ERK1/2 in the hippocampus. β-Actin was used as loading control. (B) Histogram representing the quantitative analysis of p-ERK1/2 level normalized to ERK1/2 level. Data are expressed as means±S.E.M. n=6, $^{\#}p<0.05$ *vs.* control group, $^{*}p<0.05$ *vs.* model group.

control group, n=10, Fig. 5D) in 3 weeks. After treatment with Bai (4 mg/kg) and IMI (15 mg/kg), the rearing frequencies were recovered to 8.23±2.35 and 8.9±2.53 times, respectively ($p<0.05$, n=10 *vs.* CMS model, Fig. 5D). Thus, it was shown that Bai improved the number of line crossing and rearing frequencies induced by CMS in OFT, which was similar to the effects of classic antidepressant IMI.

Bai Increases the Level of ERK1/2 Phosphorylation in the Hippocampus of CMS Rats Considering the important role of ERKs in depression, the total and phosphorylation ERK1/2 were measured by Western blotting. As shown in Fig. 6A, the results indicated that there were no significant differences in the total levels of hippocampal ERK1/2 among all groups. However, the level of phosphorylated ERK1/2, the active form of ERK1/2, was significantly decreased in CMS group. The reduction of phosphorylated ERK1/2 was alleviated by Bai and IMI (Fig. 6B). Meanwhile, Bai and IMI significantly increased pERK level when compared with CMS group, indicating that similar to that of IMI, Bai markedly increased the phosphorylation of ERK1/2 level in

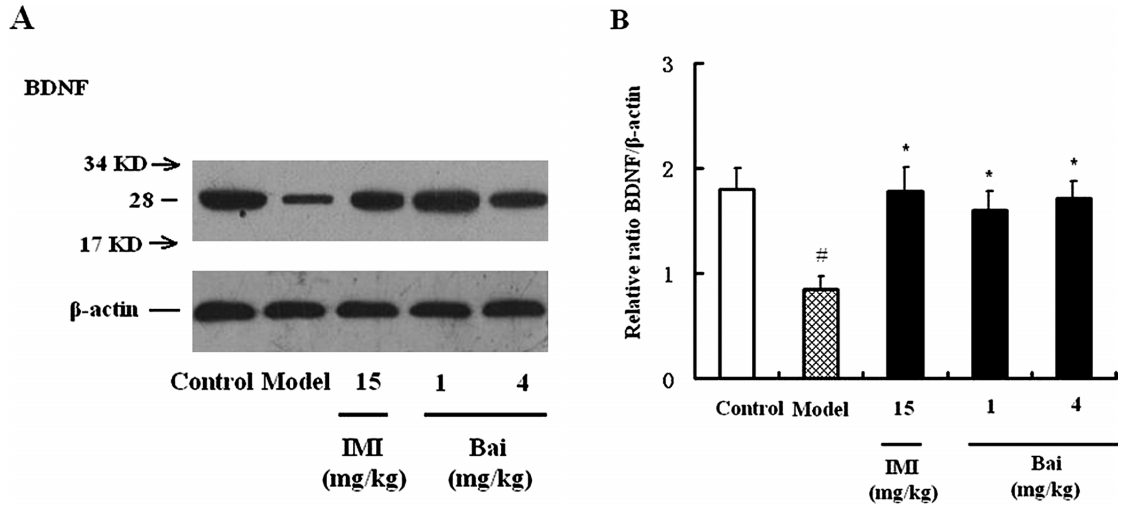

Fig. 7. Bai Recruites BDNF Expression in the DG Area of Hippocampus in CMS Rats

(A) Western blot analysis indicating the changes in BDNF in the hippocampus. β-Actin was used as loading control. (B) Histogram representing the quantitative analysis of BDNF level normalized to β-actin level. Data are expressed as means±S.E.M. $n=6$, $^{\#}p<0.05$ *vs.* control group, $^{*}p<0.05$ *vs.* model group.

CMS rats. This increased pERK may secondarily produce a recovery of hippocampal ERK-mediated neurotrophic action after chronic mild stress treatment, and at least partially, contribute to the antidepressant effect of Bai.

Bai Recruits BDNF Expression in the DG Area of Hippocampus in CMS Rats BDNF, an upstream signaling factor of ERKs, is attributed to its critical role in the development of CNS and the decrease in its expression results in neuronal damage and the inhibition of neurogenesis, especially in the DG area of hippocampus. As shown in Fig. 7A, BDNF expression in the DG area of hippocampus was decreased in CMS model. On the contrary, after treatment with 1 mg/kg and 4 mg/kg Bai, the expression of BDNF was gradually reversed (Fig. 7B). This effect was also similar to that of classic antidepressant drug IMI. These data suggest that Bai also increases the expression of BDNF induced by CMS in the DG area of hippocampus in CMS rats.

DISCUSSION

In the present study, we show that a plant-derived flavonoid Bai has antidepressant-like effects in rats' models and its potential mechanisms. The reversal of CMS-induced suppression of hippocampal ERK-mediated neurotrophic action by Bai contributes, at least partially, to the antidepressant effects of Bai. Our results suggest that stimulation of ERKs using flavonoids may confer as novel agents in the treatment of depression in the future.

Both FST and TST have been widely used to assess new antidepressant drugs activity in the preclinical study.[23,26] In this study, we show that acute administration of Bai might have a possible for antidepressant tendency because Bai reduce the immobility time at doses of 1, 2, 4 mg/kg in TST and FST of mice. Considering that the mood elevating effect of antidepressant appears often after a few weeks of treatment in clinic and most studies of antidepressant drug used rats in CMS model, Bai was administrated for 7 consecutive days to investigate the antidepressant-like property in FST of rats. Interestingly, Bai still produced inhibitory effect on the duration of immobility, with the similar potency to the classic antidepressant drug IMI. These results suggest that Bai might be a potential novel antidepressant. To further confirm the possibility, the CMS model, which was generally thought to be the most promising and valuable rodent model to study depression in animals, was selected in order to mimic several human depressive symptoms. Previous report by Willner CMS also convinced that CMS appeared more suitable for studying the neurobiological basis of depression and the effect of antidepressant drugs.[30] In present study, the results show that the immobility time induced by CMS in FST of rats was increased and this deficit was effectively reversed by chronic Bai treatment. In addition, in open field test, when the rats were placed in a novel environment, the decrease in locomotor activity (including the locomotion and rearing frequencies) was observed, which were consistent with the FST results of CMS model group, indicated a possible role of Bai in the treatment of depression. In this study, we selected three doses (1, 2, 4 mg/kg) of Bai to investigate the antidepression effect due to our previous study showed that 4 mg/kg Bai could markedly attenuate neuron injury induced by cerebral ischemia.[13] It was found that Bai decreased the immobility time and improved the scores of open field test compared to control and CMS group in the acute and chronic administration, although the dose-dependent curve was not obvious. However, considering that there is lack of dose-dependent antidepressant effect of Bai in the current study, we will prolong administration duration with higher dosage of Bai to determine its dose-dependent antidepressant effect in the next experiment. Moreover, rats or mice without stress in open field test showed that Bai exhibited little behavioral damage because the locomotor activity caused by Bai treatment at 1 mg/kg, 2 mg/kg, 4 mg/kg was similar to that of control group. It has been demonstrated that some single compounds which are widely existed in the plants,[17,18] such as curcumin and quercetin, exhibit slow, mild and lasting antidepressant effects. Considering Bai as a flavonoid extracted from traditional herb and the treatment of depression needs a relative long period, it is believed that Bai may display potential advantages in the treatment of depression.

In recent years, the biological research of depression has

been focused on neuroprotective effect and neurogenesis in the dentate gyrus of hippocampus.[31,32] It is well accepted that impairment of cell proliferation and neurogenesis in the hippocampus of adult mice underlines the pathology of depression and drug or strategy that reverse these defecits exerts commendable effects in depression treatment.[33] ERKs signal pathway is associated with neurotrophic action, cell survival and proliferation.[11] Furthermore, it has been shown that acute administration of a MAPK pathway inhibitor increases depressive-like behavior and blocks the behavioral action of antidepressants in mice.[34] Thus, the activity of ERKs might be an important index in the action of antidepressants and the reduction of ERKs phosphorylation caused by CMS may play a pathological role in depression. In present study, we show that after chronic treatment of Bai, the CMS-mediated reduction of ERK1/2 phosphorylation was significantly recovered, though at the dose of 4 mg/kg, the level of ERK1/2 phosphorylation was lower than that at the dose of 1 mg/kg. These results are consistent with previous reports that a prevalent activation of ERKs by chronic antidepressants. BDNF, one of the ERKs upstream signal factor, initiates TrkB receptor-dependant activation of ERKs and has been reported to provide a series of neuroprotective effects and neurogenesis in CNS. On the other hand, BDNF promotes the activation of CREB (cAMP-response element binding protein) through kinds of signal molecule and upregulates BDNF expression. Thus, BDNF is both an upstream regulator and downstream regulator of ERK cascade. The decrease in the expression of BDNF resulted in the production of excessive free radical, intracellular Ca^{2+} overload and neuron injury.[35,36] Some studies for antidepressant treatment have shown that electroconvulsive produced antipressant action *via* alterations of brain concentrations of neurotrophic factors by its stimulation.[37] Thus, the increase in BDNF expression appears to be of special interest in the treatment of depression. In the present study, Western blotting experiment demonstrated that BDNF level was lowered in the hippocampus of CMS rats and chronic Bai treatment increased the hippocampal level of BDNF, suggesting that BDNF may participate in the antidepressant effect of Bai, which was consistent with the result of pERKs. These data further proved that the antidepressant effect of Bai was at least partly correlated with the signaling pathway of ERK.

Our present study indicates that Bai exerts antidepressant-like effects in experimental animal models and the antidepressant mechanism may be related to the modulation the signaling pathway of ERK in the hippocampus. Considering its diverse CNS pharmacological profiles and clinical application with low toxicity, Bai may serve as a potential agent for depression treatment in the future.

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