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Research Paper

Cinnamic aldehyde treatment alleviates chronic unexpected stress-induced depressive-like behaviors via targeting cyclooxygenase-2 in mid-aged rats

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ABSTRACT

Ethnopharmacological relevance: COX-2 has been considered as a potent molecular target for prevention and therapy of depression. However, a recent study showed that COX-2 inhibitor does not improve depressive symptoms in persons aged 70 and over. Therefore, whether treatments targeting COX-2 have a clinical efficacy in depression, especially elderly individuals, remains unclear. Cinnamic aldehyde is a major constituent of *Cinnamomum cassia*, which has exhibited excellent anti-inflammatory activities as a COX-2 inhibitor. To investigate the potential antidepressant effect of cinnamic aldehyde in mid-aged rats.
Materials and methods: The depressive-like behaviors were measured after the rats exposed to chronic unexpected mild stress (CUMS). Cinnamic aldehyde was administrated by oral gavage to stressed rats (22.5, 45, 90 mg/kg, respectively) for 21 days. The mRNA, protein expression and activity of cyclooxygenase-2 (COX-2), as well as prostaglandin E_2 (PGE_2) levels were measured in the frontal cortex and hippocampus of stressed animals.
Results: We found that CUMS procedure not only decreased the sucrose preference, but also elevated the COX-2 activity, mRNA and protein levels, and increased PGE_2 concentration in rat brain regions. Treatment with high doses of cinnamic aldehyde (45, 90 mg/kg) reversed the behavioral abnormalities, and decreased the COX-2 protein and activity (but not COX-2 mRNA expression) and PGE_2 concentration in frontal cortex and hippocampus of stressed rats.
Conclusion: Cinnamic aldehyde exerted antidepressant-like effects in stressed mid-aged rats, and its mechanism of action appears to decrease COX-2 protein and activity. The current findings suggest that targeting COX-2 system might be benefit to the depression, especially elderly individuals and cinnamic aldehyde might be a promising medicine to treat the subjects in the depression.

1. Introduction

Depression may become one of the principal leading causes of disease burden by 2030 according to the World Health Organization (WHO). Thus, the discovery of antidepressants is urgent and attractive, and could arouse considerable impacts on worldwide (Lee et al., 2014). Monoamines play an important role in the pathophysiology of depression, and recent treatment chosen for depression have mainly focused on medications which change the activity of monoamine neurotransmitter systems (Hasler, 2010). However, the monoaminergic theory of illness has failed to find novel antidepressants beyond the limited therapeutic methods currently available (Brigitta, 2002). There is now a clear body of recent evidence to support an etiologic role for other factors in the pathophysiology of depression, especially activation of the inflammatory system (Pace et al., 2007; Schiepers et al., 2005). Serum proinflammatory cytokines are increased in individuals with major depression and levels of these cytokines may reflect depression severity (Duman and Voleti, 2012; Szelenyi and Selmeczy, 2002).

One inflammatory pathway implicated in major depression is the conversion of arachidonic acid (AA) to prostaglandins by the enzyme cyclooxygenase (COX). COX exists in two types of isoforms: COX-1, which is stably expressed and is cytoprotective; and COX-2, which is inducible by cytokines and promotes further inflammation (Ricciotti and FitzGerald, 2011). Clinical trails have showed that adjunctive COX-2 inhibition with celecoxib is beneficial in treating depression (Muller et al., 2006; Nery et al., 2008). We also found celecoxib reverses chronic unpredictable stress-induced depressive-like behaviors in rats

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(Guo et al., 2009). Therefore, treatments targeting COX-2 should have a clinical efficacy in depression. However, a recent study showed that COX-2 inhibitor does not improve depressive symptoms in persons aged 70 and over (Fields et al., 2012). Therefore, whether treatments targeting COX-2 have a clinical efficacy in depression, especially elderly individuals, remains unclear.

Cinnamomum cassia (Lauraceae) is a kind of deciduous tree that grows in Korea, China, and Japan, which has long been ethnopharmacologically used as a folk medicine to treat various inflammatory diseases (Seo et al., 2005; Yu et al., 2012). Its extracts contain several active components such as cinnamic aldehyde, cinnamic alcohol, cinnamic acid, and coumarin. As a major and effective compound isolated from Cinnamomum cassia, cinnamic aldehyde decreased carrageenan-induced COX-2 expression in the edema paw (Liao et al., 2012). We also found that cinnamic aldehyde reduced IL-1β-induced COX-2 activity in rat cerebral microvascular endothelial cells. These findings suggest that cinnamic aldehyde has excellent anti-inflammatory activities as a COX-2 inhibitor (Guo et al., 2009).

Based on the above foundings, in the present study we investigated the effects of cinnamic aldehyde in 18 months old rats following by 21 days chronic unpredictable mild stress (CUMS). Considering the life-span of Sprague-Dawley rats is 2.5–3.5 years, 18 months old rats is roughly equivalent to the human age of 50 to 55 (mid-age) (Shi, 1999). Cinnamic acid was orally administered to the stressed mid-aged rats for 21 days during the CUMS treatment. The potential antidepressant effect of cinnamic aldehyde was then clarified by sucrose preference test and open field test. In addition, levels of COX-2 activity, protein, and mRNA, and prostaglandin E_2 (PGE_2, a major COX-2-mediated inflammatory mediator) concentration in frontal cortex and hippocampus were also detected.

2. Methods and materials

2.1. Materials

Cinnamic aldehyde (99%) was provided by School of Pharmaceutical Sciences in Peking University, which was isolated from *Cinnamomum cassia*. Fluoxetine hydrochloride was from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, PR China). PGE_2 enzyme-linked immunosorbent assay (ELISA) kit was purchased from Invitrogen Biomedical Co. (Carlsbad, CA). COX ELISA kit was purchased from Cayman Chemical Co. (Ann Arbor, MI). TRIzol, Reverse Transcription Reagents, SYBR Green PCR Master Mix and electrophoresis reagents were obtained from Takara Co. (Tokyo, Japan). All other materials were purchased from Sigma Co. (St. Louis, MO).

2.2. Animals

Male Sprague-Dawley rats aged 18 months were obtained from Beijing Weitong Lihua Research Center for Experimental Animals. Rats were kept on a 12:12 h in the light: dark cycle (lights on at 7:00 A.M., lights off at 7:00 P.M.) in individual home cages with food and water available *ad libitum* except as described in stress.

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care of the European Community guidelines (EEC Directive of 1986; 86/609/EE). Experimental procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Psychology of the Chinese Academy of Sciences. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.3. Groups and treatment

Sixty rats were equally assigned into five groups: chronic unpredictable mild stress group, cinnamic aldehyde groups (22.5, 45 and 90 mg/kg) and fluoxetine group. The groups and treatments are shown in Table 1. Animals were administrated with corresponding drugs for 21 days during the 21-day exposure to CUMS. Twelve separate rats administrated with an equal volume of distilled water were used as a control. Fluoxetine and cinnamic aldehyde were diluted in distilled water and given one hour before the stress exposure. All drugs were prepared immediately before use and were given orally in a volume of 0.5 mL/100 g body weight with a rat gavage needle (length 10 cm).The treatment protocol of doses and administration route used for cinnamic aldehyde and fluoxetine were adopted according to previous studies (Lou et al., 2010; Song et al., 2013).

2.4. Chronic unpredictable stress procedure

The chronically stress procedure was described previously (Guo et al., 2009). Briefly, rats were exposed to different stressors daily for 21 days as follows. day1: cold immobilization for 1 h at 4 °C, forced swim for 30 min at 25 °C; day 2: immobilization for 1 h, crowding for 23 h; day 3: forced cold swim stress for 5 min at 10 °C, isolation (Each rat was placed in a closed chamber) for 23 h; day 4: immobilization for 1 h, vibration for 1 h; day 5: forced swim stress for 30 min at 25 °C, cold immobilization for 1 h at 4 °C; day 6: forced cold swim stress for 5 min at 10 °C, crowding (Six rats were placed in a cage) for 23 h; day 7: vibration for 1 h, isolation for 23 h. This schedule was repeated twice for a total of 21 days. Prior to the study, certain criteria were set for excluding animal on weight loss, or the possible occurrence of wounds. Rats were acclimated to 3 min of handling once a day for 7 consecutive days before being used in experiment and were weighed on the 1st and 7th day of handling.

2.5. Sucrose preference test

Sucrose preference tests were used to operationally define anhedonia. Specifically, anhedonia was defined as a reduction in sucrose intake and sucrose preference relative to the intake and preference of the control group. A sucrose preference test consisted of first removing the food and water from each rat's cage for a period of 20 h. Water and 1% sucrose were then placed on the cages in preweighed glass bottles, and animals were allowed to consume the fluids freely for a period of 1 h. Two baseline preference tests were

Table 1
Groups and treatments.

Groups	Treatment	Drug	Dose (mg/kg)
Control	No treatment	Distill water	–
CUMS	CUMS	Distill water	–
CUMS + Cinnamic aldehyde (22.5 mg/kg)	CUMS	Cinnamic aldehyde	22.5
CUMS + Cinnamic aldehyde (45 mg/kg)	CUMS	Cinnamic aldehyde	45
CUMS + Cinnamic aldehyde (90 mg/kg)	CUMS	Cinnamic aldehyde	90
CUMS + fluoxetine (10 mg/kg)	CUMS	Fluoxetine	10

performed, separated by at least 5 days, and the results were averaged. A preference test was also conducted following the 21 days chronic unpredictable stress period. On the last-stressed day, rats were deprived of water and food for 20 h, then from the next day on, rats were given a 1 h window sucrose preference test (24 h after the last drug treatment). Sucrose and water consumption (ml) was measured and the sucrose preference was calculated as the sucrose preference (%)=sucrose consumption /(sucrose consumption+water consumption).

2.6. Open field exploratory behavior test

Open field test was used to study the exploratory and anxiety behavior of rats and was performed after the sucrose preference test. The open field apparatus consisted of a square arena 60 × 60 cm with 40 cm high wall. The entire apparatus was painted black except for 6 mm white lines that divided the floor into 16 equal size squares. The squares were subdivided into peripheral and central sector, where the central sector included the 4 central squares (2 × 2) and the peripheral sector contained the squares close to the wall. The apparatus was illuminated with a low intensity diffuse light (45 W, 200 lux in the center area) situated 45 cm above the floor level. Entire room, except the open field, was kept dark during the experiment. Each animal was placed in the central square and observed for 5 min by a video camera and taped for further analysis. Motility was scored when an animal crossed a sector border with both its hind-limbs. The following behaviors were scored by an observer who was blind to the drug treatment. Central ambulation: number of central squares crossed; Total ambulation: the overall number of peripheral and central square crossed; rearing: number of times the animal stood on its hind limbs; grooming: number of times the animal made these responses viz. grooming of the face, licking/cleaning and scratching the various parts of the body. Immobility period: the time spent immobile. Anxiety-related behavior was measured by the percentage of central ambulation and calculated as the percentage of central ambulation (%)= central ambulation/total ambulation. Between tests, the apparatus was cleaned with 5% alcohol.

2.7. Brain sample collection

After the last behavior tests, all rats were decapitated between 11:00 A.M. and 1:00 P.M. to avoid fluctuation of hormone levels. The frontal cortex and hippocampus in each hemisphere were rapidly separated on ice-plate and stored at −80 °C until analysis.

2.8. Measurement of COX-2 activity

COX activity was determined by the method of Bosetti et al. (2002) with modifications. The frontal cortex and hippocampus brain regions were homogenized in lysate buffer (10 mM Tris-HCl, pH 7.8, containing 1% Nonidet P-40, 0.15 M NaCl, and 1 mM EDTA), then chilled on ice for 30 min and centrifuged at 10,000 rpm for 15 min. Then supernatant was collected and the COX activity was determined by enzyme-linked immunosorbent assay (ELISA) as indicated in kit instructions (Cayman, Ann Arbor, MI). The kit includes isozyme-specific inhibitors for distinguishing COX-2 activity from COX-1 activity. The COX-2 activity was expressed as nmol/min/mg wet weight of tissue.

2.9. Western blotting

The frontal cortex and hippocampus were put into chilled tubes treated with an enzyme inhibitor. Brain tissue was homogenized and Western blot analysis carried out as previously reported (Guo et al., 2009), using primary antibodies for COX-2 (1:2000, Santa Cruz Biotechnology) and β-actin (1:10,000, Santa Cruz Biotechnology). A secondary antibody conjugated with horseradish peroxidase (HRP, 1:5000, Bio-Rad) was used. Immunoblots were visualized on X-ray film by chemiluminescence reaction (Pierce), and image analysis was performed on optical densitycalibrated images by AlphaEase Stand Alone software (Alpha Innotech).

2.10. Total RNA isolation and real time RT-PCR

Total RNA from frontal cortex and hippocampus was extracted using the TRIzol method, then reverse transcribed to cDNA using the Taqman Reverse Transcription Reagents. The relative COX-2 mRNA expression values were normalized to the expression value of GAPDH. The sequences for primers were: COX-2-forward 5′-TTTGTTGAGTCATTCACCAGACAGAT-3′ and reverse 5′-ACGATGTG-TAAGGTTTCAGGGAGAAG-3′(169 bp); GAPDH-forward 5′-TGAACGG-GAAGCTCACTGG-3′ and reverse 5′-GAGCTTCACAAAGTTGTCATTG-AG-3′(260 bp. PCR conditions were as follow: 95 °C for 30 s to activate the iTaq polymerase, 45 cycles of 5 s at 95 °C and 31 s at 60 °C. Melt curve was performed for all samples. Relative contents of the COX-2 genes were calculated using the ddCt method (2^{-ddCt}) as described by Schmittgen et al. (2000).

2.11. Measurement of PGE_2 concentration

Levels of PGE_2 were determined in microwaved brain extracts. Hippocampus and frontal cortex were weighed, and then extracted in 18 volumes of hexane: 2-propanol (3:2, by volume) using a glass Tenbroeck homogenizer. The prostaglandins were purified from the lipid extract using a C18 Sep-Pak cartridge (Waters). The concentration of PGE2 was determined using an ELISA kit (Invitrogen, Carlsbad, CA) and expressed as ng/g wet weight of tissue.

2.12. Statistical analysis

The results were analyzed by the Kruskal–Wallis test and Dunn's multiple comparisons test. All data are presented as the means ± standard error of the mean. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of cinnamic aldehyde on sucrose preference

Before the CUMS procedure, there was no significant difference in sucrose solution intake and sucrose preference among groups (all $P > 0.05$, data not shown). The liquid intake and preference for sucrose in six groups following 21 days of chronic unpredictable stress were shown in Fig. 1A and B. The Kruskal–Wallis test indicated that both the sucrose solution intake and the sucrose preference significantly differed among groups ($H=17.28$, $df=5$, $N=72$, $P<0.01$; and $H=22.13$, $df=5$, $N=72$, $P<0.01$). Compared with control group, rats exposed to 21-day chronic unpredictable stress exhibited a significantly decrease in sucrose preference and sucrose solution intake (both $P<0.01$), which is a key indicative of operationally defined anhedonia. Cinnamic aldehyde treatment (45, 90 mg/kg) markedly reversed the CUMS-induced decrease in sucrose preference (both $P<0.05$) and sucrose solution intake (both $P<0.05$) in a dose-dependent manner. Fluoxetine treatment also increased the stress-induced sucrose preference and sucrose solution intake (both $P<0.05$). The total liquid intake did not differ among groups (all $P>0.05$).

3.2. Effects of cinnamic aldehyde open field exploratory behavior test

Kruskal–Wallis test indicated significant differences among groups in the total ambulation ($H=27.76$, $df=5$, $N=72$, $P<0.01$), percentage of center ambulation ($H=24.71$, $df=5$, $N=72$, $P<0.01$), rearing ($H=40.31$, $df=5$, $N=72$, $P<0.01$), grooming ($H=29.14$, $df=5$, $N=72$, $P<0.01$) and immobility period ($H=43.83$, $df=5$, $N=72$, $P<0.01$). Compared with control rats, chronic stressed rats showed the significant decreases in total ambulation, percentage of center ambulation, rearing, and the marked increases in grooming and immobility period (all $P<0.01$). Cinnamic aldehyde treatment (45 mg/kg and 90 mg/kg) alleviated the CUMS-induced behavioral alterations in a dose-dependent manner, as observed by increased total ambulation ($P<0.05$ and $P<0.01$), percentage of central ambulation ($P<0.05$ and $P<0.01$), rearing ($P<0.05$ and $P<0.01$), decreased grooming (both $P<0.05$) and immobility period ($P<0.05$ and $P<0.01$). Fluoxetine treatment also affected the stress-induced behavior alterations in open field test (Table 2).

3.3. Effects of cinnamic aldehyde on COX-2 activity

The Kruskal–Wallis analysis indicated that COX-2 activity significantly differed among groups (Frontal cortex: $H=25.53$, $df=5$, $N=72$, $P<0.01$; Hippocampus: $H=21.20$, $df=5$, $N=72$, $P<0.01$). Compared with the control group, COX-2 activity was significantly increased in frontal cortex and hippocampus after CUMS procedure (both $P<0.01$, Fig. 2). Chronic cinnamic aldehyde treatment (45 mg/kg and 90 mg/kg) reversed the stress-induced COX-2 activity both in frontal cortex ($P<0.05$ and $P<0.01$, respectively) and hippocampus ($P<0.05$ and $P<0.01$, respectively). Fluoxetine treatment slightly reduced the COX-2 activity in stressed rats (Frontal cortex: $P=0.062$; Hippocampus: $P=0.071$, respectively).

3.4. Effects of cinnamic aldehyde on COX-2 protein

We further measured the COX-2 protein level in the frontal cortex (Fig. 3A) and hippocampus (Fig. 3B). Kruskal–Wallis test indicated that COX-2 protein significantly differed among groups (Frontal cortex: $H=26.30$, $df=5$, $N=72$, $P<0.01$; Hippocampus: $H=25.67$, $df=5$, $N=72$, $P<0.01$). Compared with the control rats, 21-day stressed rats showed a significant increase in COX-2 protein level in frontal cortex ($P<0.01$) and hippocampus ($P<0.01$). Chronic cinnamic aldehyde treatment (45 mg/kg and 90 mg/kg) reduced the stressed-induced COX-2 protein level both in frontal cortex ($P<0.05$ and $P<0.01$, respectively) and hippocampus ($P<0.05$ and $P<0.01$, respectively). Fluoxetine treatment also significantly decreased the

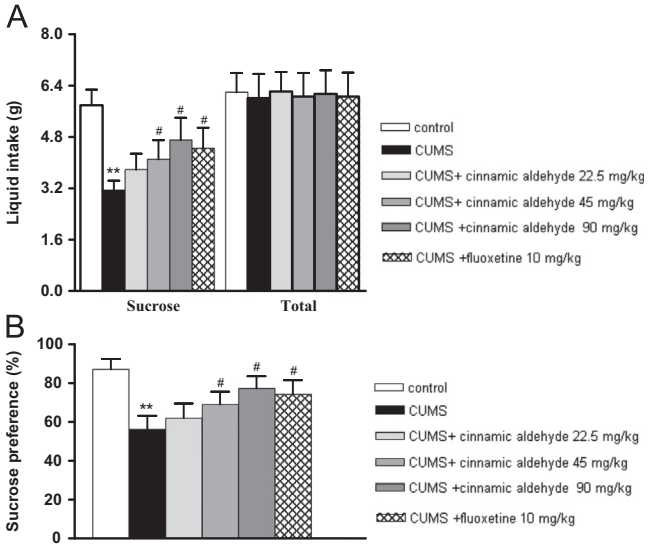

Fig. 1. Effect of cinnamic aldehyde on sucrose preference test following 21 days of chronic unpredictable stress. Sucrose solution intake and sucrose preference was reduced in the chronic unpredictable stress group compared with control group ($P<0.01$). Cinnamic aldehyde treatment significantly augmented the decrease in sucrose solution intake and sucrose preference in a dose-dependent manner. Fluoxetine treatment also increased the sucrose solution intake and sucrose preference in stressed rats. The total liquid intake did not differ among any groups. Data were expressed as mean ± SEM ($n=12$). * and # indicates statistical significance in comparison to control and chronic unpredictable stress groups respectively and denotes *,# $P<0.05$ and **, ## $P<0.01$.

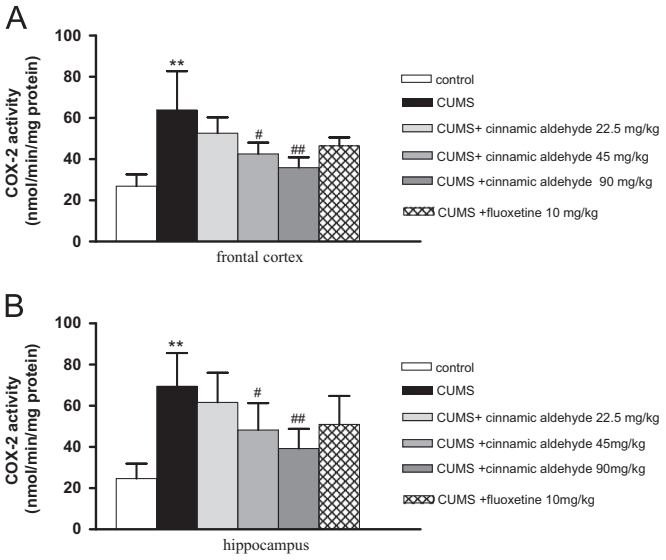

Fig. 2. Effect of cinnamic aldehyde on brain COX-2 activity following 21 days of chronic unpredictable stress. The COX-2 activity was significantly increased both in frontal cortex (A) and hippocampus (B) after chronic unpredictable stress ($P<0.01$). Cinnamic aldehyde treatment significantly reduced the stress-induced COX-2 activity in a dose-dependent manner. Fluoxetine treatment slightly reduced the stress-induced COX-2 activity ($P>0.05$). Data were expressed as mean ± SEM ($n=12$). * and # indicates statistical significance in comparison to control and chronic unpredictable stress groups respectively and denotes *,# $P<0.05$ and **, ## $P<0.01$.

Table 2
Effect of cinnamic aldehyde on open field exploratory test in chronic unpredictable stress rats.

Groups	Total ambulation	Central ambulation (%)	Rearing	Grooming	Immobility period (s)
Control	39.3 ± 6.6	32.1 ± 5.1	21.9 ± 2.8	5.2 ± 1.6	100.5 ± 15.1
CUMS	21.8 ± 3.7[b]	17.1 ± 4.1[b]	12.4 ± 1.6[b]	10.1 ± 1.9[b]	232.6 ± 36.2[b]
CUMS + Cinnamic aldehyde (22.5 mg/kg)	25.1 ± 3.5	21.3 ± 4.1	15.2 ± 2.3	9.3 ± 1.2	207.1 ± 36.9
CUMS + Cinnamic aldehyde (45 mg/kg)	29.3 ± 4.3[c]	24.8 ± 4.2[c]	17.2 ± 2.4[c]	7.0 ± 1.6[c]	179.1 ± 26.7[c]
CUMS + Cinnamic aldehyde (90 mg/kg)	32.6 ± 8.8[d]	30.1 ± 3.7[d]	20.5 ± 1.9[d]	6.4 ± 1.1[c]	159.3 ± 20.9[d]
CUMS + fluoxetine (10 mg/kg)	30.5 ± 4.9[c]	25.4 ± 4.1[c]	18.0 ± 1.5[c]	6.2 ± 1.1[c]	177.1 ± 20.1[c]

Data are expressed as mean ± S.E.M of 12 rats per group. [a, b] Significantly different from control group value ([a], $P<0.05$; [b], $P<0.01$). [c, d] Significantly different from chronic unpredictable stress group value ([c], $P<0.05$; [d], $P<0.01$).

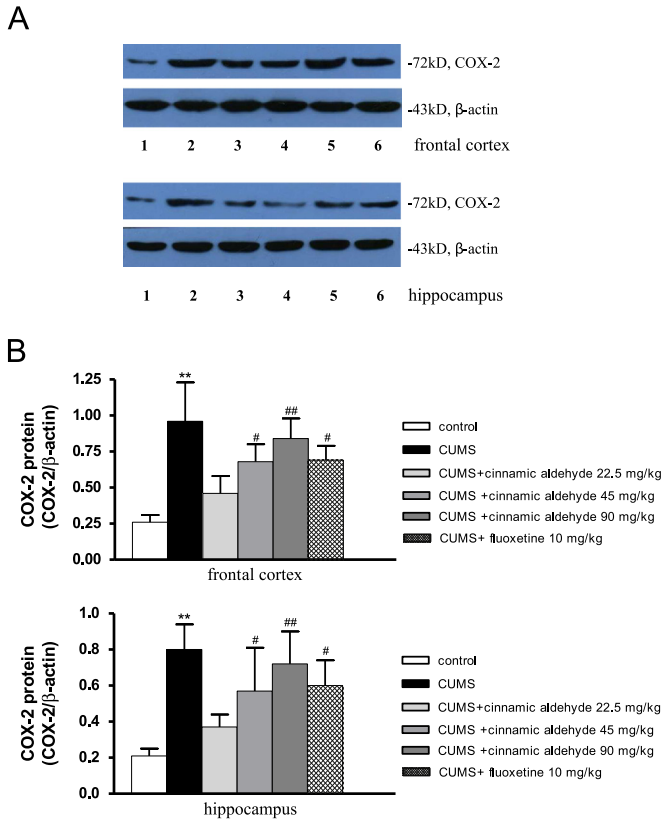

Fig. 3. Effect of cinnamic aldehyde on brain COX-2 protein level following 21 days of chronic unpredictable stress. A. Representative immunoblots of COX-2 and β-actin in prontal cortex and hippocampus. 1, control; 2, chronic unpredictable stress; 3, chronic unpredictable stress plus cinnamic aldehyde (22.5 mg/kg); 4, chronic unpredictable stress plus cinnamic aldehyde (45 mg/kg); 5, chronic unpredictable stress plus cinnamic aldehyde (90 mg/kg); 6, chronic unpredictable stress plus fluoxetine (10 mg/kg). B. Relative optical density (OD) of COX-2 to β-actin in prontal cortex and hippocampus. Data were expressed as mean ± SEM ($n=12$).* and # indicates statistical significance in comparison to control and chronic unpredictable stress groups respectively and denotes *,# $P<0.05$ and **, ## $P<0.01$.

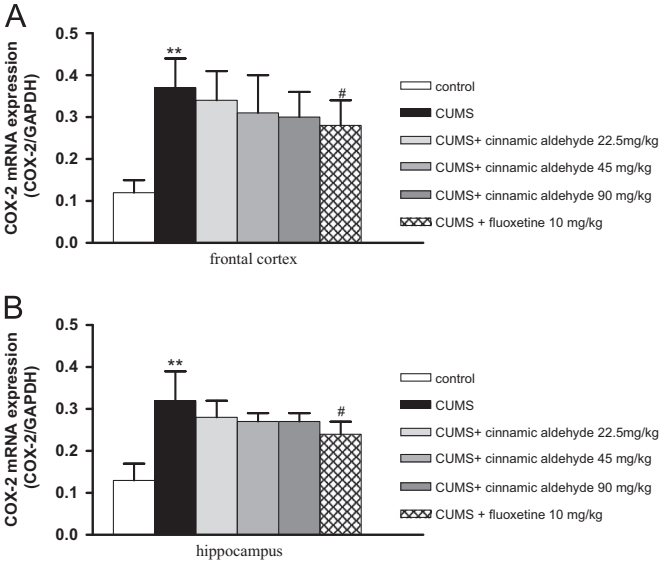

Fig. 4. Effect of cinnamic aldehyde on brain COX-2 mRNA expression following 21 days of chronic unpredictable stress. The COX-2 mRNA expression in chronic unpredictable stress group was significantly increased both in frontal cortex (A) and hippocampus (B) than these in the control level ($P<0.01$). Cinnamic aldehyde treatment did not affect the stress-induced upregulation of COX-2 mRNA. Fluoxetine treatment significantly reduced COX-2 mRNA expression in stressed rats ($P<0.05$). Data were expressed as mean ± SEM ($n=12$).* and # indicates statistical significance in comparison to control and chronic unpredictable stress groups respectively and denotes *,# $P<0.05$ and **, ## $P<0.01$.

COX-2 protein level in the stressed rats (Frontal cortex: $P<0.05$; Hippocampus: $P<0.05$, respectively).

3.5. Effects of cinnamic aldehyde on COX-2 mRNA expression

COX-2 mRNA expression was also detected in the frontal cortex and hippocampus. As shown in Fig. 4 A and B, the Kruskal–Wallis test revealed that COX-2 protein significantly differed among groups (Frontal cortex: $H=18.29$, $df=5$, $N=72$, $P<0.01$; Hippocampus: $H=20.52$, $df=5$, $N=72$, $P<0.01$). Compared with the control group, COX-2 mRNA expression in the stressed rats was significantly increased about 4 times in the frontal cortex and hippocampus after CUMS procedure (both $P<0.01$). However, cinnamic aldehyde treatment did not affect the stress-induced upregulation in COX-2 mRNA expression ($P>0.05$). Fluoxetine treatment significantly reduced the COX-2 mRNA expression in stressed rats (Frontal cortex: $P<0.05$; Hippocampus: $P<0.05$, respectively).

3.6. Effects of cinnamic aldehyde on PGE_2 levels

The effects of cinnamic aldehyde and fluoxetine on PGE_2 content in the frontal cortex and hippocampus were shown in Fig. 5A and Fig. 5B. The Kruskal–Wallis test indicated that PGE_2 level significantly differed among groups (Frontal cortex: $H=20.49$, $df=5$, $N=72$, $P<0.01$; Hippocampus: $H=19.75$, $df=5$, $N=72$, $P<0.01$). Compared with control, stressed rats showed a significantly increase in PGE_2 concentration both in frontal cortex ($P<0.01$) and hippocampus ($P<0.01$). Chronic treatment with 45 mg/kg and 90 mg/kg cinnamic aldehyde significantly attenuated CUMS-induced increase in PGE_2 both in frontal cortex ($P<0.05$ and $P<0.01$, respectively) and hippocampus ($P<0.05$ and $P<0.01$, respectively). Fluoxetine also markedly reduced the stress-induced PGE_2 concentration both in frontal cortex and hippocampus (both $P<0.05$).

4. Discussion

In the present study, we investigated the effects of cinnamic aldehyde on depression-like behaviors in the mid-aged rats exposed to 21-day CUMS. The sucrose preference and open field exploratory tests were used to assess the depression levels in animal. After the behavioral test, the COX-2 mRNA, protein expression and activity as well as PGE_2 concentration in the hippocampus and frontal cortex were measured. The results showed that cinnamic aldehyde treatment at a daily dosage of 45 and 90 mg/kg but not 22.5 mg/kg exhibited a potential antidepressant effect on CUMS-produced depression in mid-aged rats. At the meanwhile, cinnamic aldehyde led to a decrease in COX-2 activity reactivity, protein expression and PGE_2 concentration without any effect on the COX-2 mRNA level in the CUMS-treated rats.

Depression is the most common psychiatric disease in the elderly, with a prevalence ranging from 22 to 46% in patients over 60 years old (Lebowitz et al., 1997). This phenomenon is getting attention recently since the world's geriatric population is importantly increasing (Mendlewicz, 1998). In the previous study Herrera-Pérez et al. found that a higher percentage of middle-aged rats developed anhedonia as compared with young adult ones (Herrera-Perez et al., 2008). Of particular interest to this study is aim to investigate antidepressant effect of cinnamic aldehyde in mid-aged rats. An animal model of depression in mid-aged rat followed by 21 days CUMS has been developed to simulate the pathogenesis of depression in elderly humans. However, as the rat was individually housed, there may be an overlap between isolation- and CUMS-

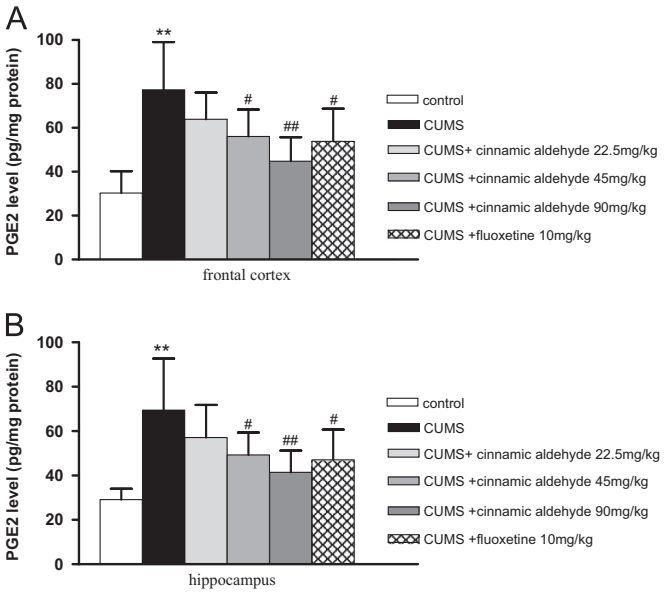

Fig. 5. Effect of cinnamic aldehyde on brain PGE_2 level following 21 days of chronic unpredictable stress. The PGE_2 concentration in chronic unpredictable stress group was significantly increased both in frontal cortex (A) and hippocampus (B) than these in the control level ($P<0.01$). Chronic treatments with cinnamic aldehyde significantly reduced the stress-induced PGE_2 concentration in a dose-dependent manner. Fluoxetine treatment also significantly reduced the stress-induced PGE_2 concentration ($P<0.05$). Data were expressed as mean ± SEM ($n=12$). * and # indicates statistical significance in comparison to control and chronic unpredictable stress groups respectively and denotes *,# $P<0.05$ and **, ## $P<0.01$.

induced depression. After the CUMS paradigm, behavioral tests were performed by the sucrose preference and open field tests. The sucrose preference test is an indicator of anhedonia-like behavioral change, which a key symptom of major depression among humans (Willner, 1997). As described in the result section, stressed rats consumed less sucrose solution compared to control rats. Rats followed by 21-day chronic unpredictable stress also showed anxious behaviors which is proved by decreased ambulation, rearing, increased grooming and immobility period in the open field exploratory test.

Inflammation has been considered as a biological risk factor for late-life depression (Krishnan, 2002). Prostaglandins, especially PGE_2, play an important role in the generation of the inflammatory response. The biosynthesis of PGE_2 is significantly increased in various inflamed tissues, and which contributes to the development of the important signs of inflammation (Ricciotti and FitzGerald, 2011). COX is a rate-limiting enzyme in the metabolism of arachidonic acid to prostaglandins. COX-2 as the key inflammatory enzyme was increased in chronic stress-induced young rat in the previous study (Guo et al., 2009). However, whether COX-2 expression was also increased in the aged depression animal remains unclear. Recent clinical and animal studies of depression have focused on the brain region of frontal cortex and hippocampus, which are key brain regions structurally and functionally influenced by stress responses and critically involved in the regulation of mood and learning/memory function (Hastings et al., 2004; Rocher et al., 2004). In the present study, we first study the COX-2 expression in the hippocampus and frontal cortex in chronic stress-induced middle aged rat. We found that the protein, mRNA and activity of COX-2 were increased in chronic stress-induced mid-aged rats. In addition, COX-1 activity was also measured in the present study. We found that CUMS procedure did not affect COX-1 activity (data not shown).The concentration of PGE_2, a bioactive metabolite of arachidonic acid (AA) induced by COX enzyme, was also enhance by CUMS treatment in the hippocampus and frontal cortex regions.

Cinnamomum cassia is a representative ethnoherbal medicine that has been widely used for treating dyspepsia, gastritis, and other inflammatory diseases (Hong et al., 2002). Cinnamic aldehyde is a major constituent of *Cinnamomum cassia*, which is also widely distributed in various fruits, vegetables and flowers. Cinnamic aldehyde has been known to have various biological activities including anti-inflammatory, antioxidant and anti-bacterial properties (Youn et al., 2008). To elucidate the mechanisms underlying the potential antidepressant effects of cinnamomum cassia, we observed the changes of liquid intake, sucrose preference, behavioral alterations, COX-2 expression, PGE_2 concentration in chronic unpredictable stress plus chronic cinnamomum cassia treatment in the mid-aged rat. The results revealed that chronic cinnamomum cassia treatment could elevate the stress-induced decrease in sucrose solution intake, sucrose preference, and reverse the stress-induced behavioral alterations in a dose-dependent manner. We also found that chronic cinnamic aldehyde treatment reduced COX-2 protein and its enzymatic activity but not its mRNA level in rat brain. Furthermore, chronic cinnamic aldehyde treatment reduced the brain concentration of PGE_2, thus down-regulating a key downstream step in the AA cascade. COX-2 mRNA possesses 'AUUUA' motifs in its 3'-untranslated region that produce post-transcriptional control of COX-2 expression by acting as translation inhibitory element or as mRNA instability determinant (Dixon et al., 2000; Kujubu et al., 1991). Many 'instable' messages in the 3'-untranslated region carry motifs that might control translational efficiency via reversibly binding to nuclear or cytosolic factors (Bosetti et al., 2002; Hla and Neilson, 1992). Cinnamic aldehyde might directly bind to COX-2. Several residues have been reported to be involved in binding of selective COX-2 inhibitors, such as His90, Arg120, Phe518, Tyr385, and Ser530 (Murias et al., 2004). Cinnamic aldehyde might bind to one of these binding sites and affect COX-2 mRNA stability and translation. Chronic cinnamic aldehyde treatment, either directly or indirectly, could affect the interactions between these RNA and protein. Cinnamic aldehyde's post-transcriptional effect on COX-2 system could also be mediated by reduced formation of the PGE_2 that mitigates COX-2 mRNA decay and inhibition of protein translation, normally mediated by the 3'-untranslated region of COX-2 mRNA.

To reduce the number of animals used, the effect of cinnamic aldehyde on control rats was not investigated in the present study. Antidepressants usually do not induce any significant changes on behaviors in control animals which the depression have not yet been developed. Furthermore, the key symptom of depression is anhedonia and sucrose preference test was alwasys used to operationally define anhedonia. The values of sucrose preference in unstressed control animals are normally about 90% and these values are very hard to be elevated further by the drug treatment on unstressed control animals. These results have been showed in our and other studies (Guo et al., 2009; Mao et al., 2011).

The current findings support the previous results that targeting cyclooxygenase-2 is a viable therapeutic approach for depression (Li et al., 2013; Muller et al., 2006). However, Fields et al. reported a randomized controlled trial of NSAID treatment in over 2500 older adults, the results showed that COX-2 inhibitor celecoxib could not improved depressive symptoms over time compared with placebo. Possible reasons for the discrepancy may that the dosage of COX-2 inhibitor used by Fields et al. (2012) may not reach the therapeutic level. Alternatively, the mid-aged rats but not aged rats may sensitive to COX-2 inhibitor treatment. Considering COX-2 expression may change over the course of life, it will be very interesting to measure this protein level in immature, mature, middle-aged and aged rats and then analyze the effect of cinnamic aldehyde in depression rat at the different age. Besides, it should be noted that PGE2 is a key mediator of inflammation in the depression. Other

proinflammatory cytokines such as interleukin(IL)-1β, IL-6 and tumor necrosis factor (TNF)-α are also increased in CUMS rat and depressive symptoms in human (Frommberger et al., 1997; Liu et al., 2014). The effect of cinnamic aldehyde on these cytokines deserved to be measured in the future study.

In conclusion, the present study investigated the antidepressant-like effects and the potential mechanisms of cinnamic aldehyde in the CUMS model of mid-aged rats. The CUMS procedure elevated COX-2 activity, protein and mRNA expression, as well as its catalysate PGE_2 level in the frontal cortex and hippocampus regions. Chronic cinnamic aldehyde treatment deceased COX-2 protein and COX-2 activity but not COX-2 mRNA expression, subsequently resulted in reduction of PGE_2 levels in brain. These findings indicate that cinnamic aldehyde treatment alleviates the depression-like behavior via the COX-2 system in brain in mid-aged rats. Our results suggest that targeting COX-2 system might be benefit to the depression, especially elderly individuals.

Authors' contributions

Guo designed the study and drafted the manuscript. Yao, Huang and Yang conceived of the study. All authors read and approved the final manuscript.

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