

Antidepressant-like Effect of EGb 761 in Rats with Chronic Mild Stress-induced Depressive Behaviours

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ABSTRACT

Objective: The investigated the effects of EGb 761 on depressive behaviours in rats exposed to chronic mild stress (CMS) and the possible mechanisms of the actions.

Methods: Wistar rats were randomly divided into normal, model and EGb 761 groups. Animals in the model and EGb 761 groups were exposed to a CMS procedure lasting 28 days, while simultaneously giving EGb 761 by gastric administration daily to rats in EGb 761 group for 28 days. Behavioural alterations were investigated by open-field-test, sucrose preference test and forced swim test (FST). Levels of IL-1 β , IL-6 and vascular endothelial growth factor (VEGF) in homogenates of the hypothalamus and pituitary gland were measured by ELISA Serum levels of corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol (CORT) were detected by radio-immunoassay.

Results: Chronic mild stress decreased locomotor activity and sucrose consumption, prolonged immobility duration in FST, elevated levels of IL-1 β , IL-6, CRH, ACTH and CORT, and reduced levels of VEGF in the rats from the model group, when compared to the normal group. However, EGb 761 treatment ameliorated the CUMS-induced alterations in sucrose consumption and immobility duration without affecting locomotor activity. Moreover, EGb 761 inhibited the over-production of IL-1 β , IL-6, CRH, ACTH and CORT, as well as restored the VEGF production in rats.

Conclusion: EGb 761 treatment had antidepressant-like effects in rats exposed to CMS.

Keywords: Antidepressant-like effect, chronic mild stress, depression, EGb 761, HPA axis, inflammation, VEGF

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INTRODUCTION

Depression is one of the most prevalent mental disorders and one of the leading causes of medical burden worldwide (1, 2). Besides psychotherapy, a variety of antidepressants including tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase A, selective serotonin reuptake inhibitors and some other agents were clinically used in the management of depression. However, there are still patients who cannot achieve satisfactory therapeutic effect from the above agents or suffer from adverse reactions of these drugs (3). Therefore, there is a need to discover novel agents for the management of depression.

Recently, it is gradually accepted that depression is an inflammatory state in which pro-inflammatory cytokines play an important role. Moreover, anti-inflammation is now generally regarded as a potential therapy for depression (4). Among the multiple pro-inflammatory cytokines, IL-1 β and IL-6 have well been demonstrated to contribute to the development and exacerbation of depression by inducing neuroinflammation and HPA axis dysfunctions as well as some other mechanisms (5, 6). Managements that can down-regulate the expression of IL-1 β and IL-6 were effective in ameliorating depression-like behaviours in animals with depression induced by chronic stress or LPS injection (7).

Vascular endothelial growth factor, an angiogenic and neurogenic factor, has been shown to be involved in depression. Evidence proved that depressive subjects showed reduced levels of VEGF in brain and antidepressant agents could restore the VEGF production. Interestingly, two recent studies consistently demonstrated that VEGF was necessary for the Behavioural effects of antidepressant agents such as SSRI fluoxetine, norepinephrine selective reuptake inhibitor and others (8, 9), which strongly supported the positive role of VEGF in treatment of depression. Moreover, there is also direct evidence supporting the positive role of VEGF that VEGF transgenic mice display antidepressant-like

behaviour (10). All the findings suggest VEGF might be a therapeutic target in the management of depression.

Herbal medicines and their extracts have been used as alternative or add-on therapies for depression in trials due to their variety of pharmacological activities and slight adverse reactions (11). EGb 761 is an extract from grape seed and possesses anti-inflammatory activity. It has been used in treatments of some disorders involving inflammation, such as lipopolysaccharide-induced lung injury (12), inflammatory and post-surgical pain (13). Besides, EGb 761 also showed neuroprotective effects in some recent studies, Tulsulkar J (14) very recently reported that EGb 761 exerted its neuroprotective activity by up-regulating the production of VEGF in a mouse model of transient global ischaemia. More interestingly, a study revealed that EGb761 pretreatment decreased the immobility of BALB/c mice in the forced swimming test by inhibiting oxidative stress (15). However, the potential antidepressant-like activity of EGb 761 in animals exposed to CUS has not been investigated to date. And its effects on inflammatory cytokines and VEGF in such animals are also unclear. Thus in the present study, we aimed to evaluate the effects of EGb 761 on depressive-like behaviours, IL-1 β , IL-6 and VEGF in a rat model of depression induced by CUS.

SUBJECTS AND METHODS

Animals

Twenty-four male Wistar rats (Center of laboratory animal in Shandong University, Jinan, China) weighing 220–260 grams were used in this study. The animals were allowed five days to get acclimatized to the new surroundings. Then they were randomly assigned to normal, model and EGb 761 groups with eight rats per group. After grouping, they were housed in plastic cages (four rats per cage) in a temperature-humidity- controlled (22–26 °C, 65 \pm 3%,

respectively) room, with free access to standard food and tap water and a 12:12 hour light-dark cycle unless otherwise noted. Every effort was made to minimize the suffering of the animals and the number of animals necessary for the investigations. The investigations were conducted strictly in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols described in this text were approved by the Committee on the Ethics of Animal Experiments of Anqiu People's Hospital.

Chronic mild stress procedure

Rats in the model group and the EGb 761 group were exposed to a 28 day-CMS procedure which was modified from previous studies (16). The stressors introduced in the current study include: cage tilt (45°), food deprivation (24 hours), cold swimming (4 °C), hot room (45 °C), intermittent white noise (75 Db, 2 hours), tail pinch (1 minute), physical restraint (1 hour) and wet cage (24 hours). The rat received only one stressor in a day. Rats in the normal group were left undisturbed.

EGb 761 group administration

Simultaneous with the CUS procedure, rats in the EGb 761 group were daily treated with EGb 761 (Dr. Willmar Schwabe GmbH & CO. KG) at the dose of 150 mg/kg/day (dissolved in saline) by intragastric administration for 28 days; rats in the normal group and in the model group only received equal volume of saline.

Behavioural assessments

Forced swim test

A forced swim test (FST) was performed as described previously (17). Briefly, the rat was placed in a cylinder (10 cm in diameter, 25 cm in height) filled with water (30 cm in depth, 25 ± 1 °C) and forced to swim for five minutes. The activity of the rat was video recorded to analyse the immobility duration which is a measure of depressive-like behaviour and is extensively used to assess antidepressant-like activities.

Sucrose preference test

Sucrose preference test was carried out as described previously (18) to assess anhedonia, a core symptom of major depression. Before the measurement, rats were trained to consume sucrose solution with free access to two-bottle choice of 2% sucrose solution and water for three days. After food and water-deprivation for 12 hours, rats were individually housed and presented with one bottle of 2% sucrose solution and one bottle of water for 12 h. Sucrose preference = sucrose intake/total fluid intake \times 100.

Locomotor activity test

To assess the effects of EGb 761 on locomotor activity, the locomotor activity of the animal was evaluated by open-field-test as reported previously with slight modification (19). Briefly, each rat was individually placed in an open field box (100 cm \times 100 cm \times 40 cm) consisting of 25 equal squares on the bottom to freely explore the apparatus for five minutes and the exploration was recorded by a video camera. The number of crossing which is considered as indicative of locomotor activity (squares crossed with all paws) was analysed by two investigators.

Preparation of serum and tissue homogenate

After the behavioural test, blood samples were collected from the heart using a syringe under deep anaesthesia and centrifuged at 3000 \times g for 15 minutes to obtain serum. Then the rat was sacrificed and the hypothalamus and the pituitary gland were immediately removed from the brain. For homogenate preparation, small parts of the hypothalamus and the pituitary gland were respectively homogenized with iced-saline. The liquid supernatants of the homogenates were collected after centrifugation at 3000 \times g for 15 minutes. The serum and tissue homogenate were stored at -20 °C.

Cytokine and VEGF assessments

Concentrations of IL-1 β , IL-6 and VEGF in homogenates of the hypothalamus and pituitary

gland were measured by ELISA with commercial kits (Boster Company, Wuhan, China).

HPA axis activity assessment

To investigate the activity of the HPA axis, concentrations of CRH, ACTH and CORT in serum were detected by radioimmunoassay with commercially available kits (Beijing North Institute of Biological Technology Company, China).

Statistical analysis

Statistical analysis was performed using SPSS 16.0. Data were expressed as mean \pm standard deviation (mean \pm SD) and comparisons of the differences among the three groups were carried out using one-way ANOVA with Students-Newman-Keuls (SNK) test. It was considered statistically significant when the p -value was less than 0.05.

RESULTS

Effects of EGb 761 on behaviours of the rats

As shown in Table 1, rats in the model group presented with much longer immobility time in FST, more numbers of crossings in the open-field-test and reduced sucrose preference in the sucrose preference test than the rats in the normal group (all $p < 0.05$). EGb 761 treatment significantly reduced the immobility time ($p < 0.05$), elevated the sucrose preference ($p < 0.05$), but did not alter the number of crossings ($p > 0.05$) in the EGb 761 group if compared to the model group. The results indicated that EGb 761 could attenuate the CMS-induced depressive-like behaviours but had no significant effects on locomotor activity.

Effects of EGb 761 on levels of cytokines

Table 2 showed the concentrations of the inflammatory cytokines in the homogenates of hypothalamus and pituitary gland. Chronic mild stress significantly elevated the levels of IL-1 β and IL-6 in the hypothalamus and pituitary gland (both $p < 0.05$); however, EGb 761

treatment markedly reduced the elevation of these inflammatory cytokines if compared to the model group (both $p < 0.05$).

Effects of EGb 761 on levels of VEGF

Table 3 presents the expression of VEGF in the hypothalamus and pituitary gland of the rats. The concentrations of VEGF in the hypothalamus and pituitary gland markedly decreased in the model group as compared to the normal group (both $p < 0.05$); EGb 761 treatment remarkably reduced the reduction in VEGF production in the EGb 761 group in comparison with the model group.

Effects of EGb 761 on HPA axis activity

HPA axis activity was evaluated by the investigating the serum levels of CRH, ACTH and CORT. Much higher serum levels of CRH, ACTH and CORT were observed in the model group as compared to the normal group (all $p < 0.05$); yet, EGb 761 partially reversed the elevation of CRH, ACTH and CORT levels in serum compared to the model group (Table 4).

DISCUSSION

Here, we for the first time reported that EGb 761 produced antidepressant-like effects without affecting the locomotor activity in a rat model of depression induced by CMS. And we also found EGb 761 could inhibit productions of IL-1 β and IL-6, promote VEGF expression, as well as restore the HPA axis dysfunction in rats exposed to CMS.

Animal models are widely used to evaluate antidepressant-like activity in pre-clinical studies. In the current study, we exposed rats to CMS which is a widely employed method to induce depression in animals by some mild stressors (20). Forced swim test is a most commonly used behavioural test screening for depressive-like behaviour and evaluating antidepressant efficacy in animals (17). In the current study, we found the rats in the model

group showed significantly longer immobility time than the normal group in the FST, which meant CMS successfully induced depressive-like behaviour in rats. But EGb 761 treatment caused a marked reduction in immobility time in the EGb 761 group if compared to the model group, indicating EGb 761 significantly ameliorated the depressive-like behaviour of the animals. Yet, the forced swim test may produce “false positive” outcomes resulting from the possible alteration of locomotor activity (19). To exclude a false positive from locomotor activity caused by EGb 761, the open field test was performed. No marked effects of EGb 761 on the locomotor activity of the rats were observed in the test, which confirmed the amelioration of immobility in FST was specifically derived from the antidepressant effects. Anhedonia, *i.e.* loss of interest or pleasure, is also a symptom of depression and is usually measured by the sucrose consumption test (18). In the sucrose preference test, we found rats in the model group consumed much less sucrose solution than the normal rats, however, rats in the EGb 761 group consumed much more than the model group, suggesting EGb 761 ameliorated the CUS-induced anhedonia in rats. Thus, the finding of the three different behavioural tests consistently proved the antidepressant activity of EGb 761 in rats exposed to CUS. Because EGb 761 has many pharmacological activities (21), it is worth investigating the possible mechanism by which EGb 761 exerted its antidepressant effects.

The linking between inflammation and depression has been well demonstrated and anti-inflammation is now regarded as a potential way to treat depression (4). Studies showed CUS could elicit inflammatory response in animals. Consistently, we found CUS elevated the expression of IL-1 β and IL-6 in the hypothalamus and pituitary gland of the rats in the model group. However, treated with EGb 761, rats in the EGb 761 showed much lower levels of these inflammatory cytokines in homogenates of the hypothalamus and pituitary gland than the model group. The findings indicated that EGb 761 attenuated the neuroinflammation induced by CUS in rats. This is consistent with the earlier studies that reported the

anti-inflammatory activity of EGb 761 (22).

There is much evidence that proved VEGF contributed to the improvement of depression (8–10). In the current study, CUS significantly suppressed the expression of VEGF in the hypothalamus and pituitary gland of the rats in the model group. Yet, EGb 761 markedly reversed the reduction in the expression of VEGF in the EGb 761 group if compared to the model group. The result is in line with a previous study which reported that EGb 761 showed neuroprotective properties and elevated VEGF levels in the brain in mice with ischaemic stroke (23). We supposed that the elevation of VEGF production should contribute the amelioration of the depressive behaviours.

The activity of the hypothalamic-pituitary-adrenal (HPA)-axis is influenced by many factors in depressive subjects, for example inflammation and VEGF (24, 25). To verify whether the alterations of inflammatory cytokines and VEGF by EGb 761 were accompanied with the changes of HPA axis activity, we detected the levels of CRH, ACTH and CORT in serum. Earlier studies showed CMS could induce dysfunction of HPA axis in animals (26), and the dysfunction was associated with the development and severity of depressive-behaviours. Similarly, we found much higher serum levels of CRH, ACTH and CORT in rats from the model group if compared to the normal animals. However, treated with EGb 761, the EGb 761 group presented much lower serum levels of CRH, ACTH and CORT than the model group. The data indicated that EGb 761 could partially restore the dysfunction of HPA axis in the rats exposed to CMS.

In conclusion, EGb 761 possesses antidepressant activity in rats exposed to CMS. Besides, it can partially reverse the alterations of IL-1 β , IL-6, VEGF and HPA axis dysfunction induced by CMS in rats. The exact mechanism of the alternation of HPA axis activity needs to be further studied.

AUTHORS' NOTE

The authors report no conflicts of interest.

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Table 1: Effects of EGb761 on behaviours of the mice

	Crossing	Sucrose preference (%)	FST Immobility
Control group	66.3 ± 8.1	85.17 ± 7.31	90.08 ± 11.56
Vehicle group	45.9 ± 6.0 ^a	53.86 ± 6.53 ^a	169.13 ± 18.80 ^a
EGb761 group	46.8 ± 6.5 ^a	69.18 ± 6.40 ^{ab}	121.94 ± 13.27 ^{ab}

Data are expressed as mean ± SD ($N = 8$ per group). ^a $p < 0.05$, if compared to the control group; ^b $p < 0.05$, if compared to the model group.

Table 2: Effects of EGb761 on levels of IL-1 β and IL-6 in tissue homogenate

	Hypothalamus		Pituitary gland	
	IL-1β(pg/ml)	IL-6 (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
Control group	201.09 ± 30.36	130.62 ± 18.91	156.91 ± 23.78	113.80 ± 13.85
Vehicle group	361.52 ± 50.13 ^a	236.83 ± 30.28 ^a	298.40 ± 39.6 ^a	198.43 ± 21.17 ^a
EGb761 group	279.90 ± 36.29 ^{ab}	176.19 ± 21.15 ^{ab}	225.85 ± 33.0 ^{ab}	149.92 ± 16.05 ^{ab}

Data are expressed as mean ± SD ($N=8$ per group). ^a $p < 0.05$, if compared to the control group; ^b $p < 0.05$, if compared to the model group.

Table 3: Effects of EGb761 on levels of VEGF in hypothalamus and pituitary gland

	Hypothalamus (pg/ml)	Pituitary gland (pg/ml)
Control group	88.17 ± 6.35	78.09 ± 7.26
Vehicle group	55.03 ± 7.82 ^a	46.11 ± 5.64 ^a
EGb761 group	70.95 ± 8.41 ^{ab}	61.88 ± 8.50 ^{ab}

Data are expressed as mean ± SD ($N=8$ per group). ^a $p < 0.05$, if compared to the control group; ^b $p < 0.05$, if compared to the model group.

Table 4: Effects of EGb761 on the activity of HPA axis

	CRH (pg/ml)	ACTH (pg/ml)	CORT (pg/ml)
Control group	6.07 ± 0.53	86.73 ± 11.85	125.82 ± 9.17
Vehicle group	11.72 ± 1.71 ^a	157.11 ± 19.39 ^a	217.08 ± 18.93 ^a
EGb761 group	8.08 ± 1.10 ^{ab}	102.26 ± 14.16 ^a	159.10 ± 13.55 ^{ab}

Data are expressed as mean ± SD (*N*=8 per group). ^a*p* < 0.05, if compared to the control group; ^b*p* < 0.05, if compared to the model group.