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Emodin opposes chronic unpredictable mild stress induced depressive-like behavior in mice by upregulating the levels of hippocampal glucocorticoid receptor and brain-derived neurotrophic factor

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ABSTRACT

Emodin, the major active component of Rhubarb, has shown neuroprotective activity. This study is attempted to investigate whether emodin possesses beneficial effects on chronic unpredictable mild stress (CUMS)-induced behavioral deficits (depression-like behaviors) and explore the possible mechanisms. ICR mice were subjected to chronic unpredictable mild stress for 42 consecutive days. Then, emodin and fluoxetine (positive control drug) were administered for 21 consecutive days at the last three weeks of CUMS procedure. The classical behavioral tests: open field test (OFT), sucrose preference test (SPT), tail suspension test (TST) and forced swimming test (FST) were applied to evaluate the antidepressant effects of emodin. Then plasma corticosterone concentration, hippocampal glucocorticoid receptor (GR) and brain-derived neurotrophic factor (BDNF) levels were tested to probe the mechanisms. Our results indicated that 6 weeks of CUMS exposure induced significant depression-like behavior, with high, plasma corticosterone concentration and low hippocampal GR and BDNF expression levels. Whereas, chronic emodin (20, 40 and 80 mg/kg) treatments reversed the behavioral deficiency induced by CUMS exposure. Treatment with emodin normalized the change of plasma corticosterone level, which demonstrated that emodin could partially restore CUMS-induced HPA axis impairments. Besides, hippocampal GR (mRNA and protein) and BDNF (mRNA) expressions were also up-regulated after emodin treatments. In conclusion, emodin remarkably improved depression-like behavior in CUMS mice and its antidepressant activity is mediated, at least in part, by the up-regulating GR and BDNF levels in hippocampus.

1. Introduction

Depression, a well-known chronic, recurring, and life-threatening emotional disorder, is triggered by many environmental and genetic factors [1]. Although there are many hypotheses involved in the etiopathogenesis of depression (including monoamine deficiency hypothesis, hyperactivity of the HPA axis hypothesis, neurodegeneration and so on), the mechanism is still not very clear [2]. Especially, a majority of current clinical antidepressants only have low curative ratio and even cause side effects [3]. Therefore, looking for more effective and reliable antidepressants is necessary.

Plenty of clinical reports suggest that prolonged exposure to life stressful episodes, as a common risk factor, could provoke the development of major depression [4–6]. Currently, scientists adopt chronic unpredictable mild stress

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(CUMS) procedure which is performed such that animals are consecutively exposed to a series of unpredictable mild stressors to simulate a series of life stress events [7]. Indeed, a large number of ethological symptoms and neurobiological abnormalities found in CUMS-induced animals are similar to those exhibited in human depressed patients [8]. Thus, CUMS-induced depressive animal model has good validity and reliable predictability to screen new antidepressants through a series of behavioral tests [5].

The hypothalamic–pituitary–adrenal (HPA) axis is a key response element against stressors and its abnormal activation by chronic stressful conditions is regarded as an important risk factor for depression [6,9,10]. Previous research shows that chronic and elevated glucocorticoid levels as a result of HPA axis dysfunction existed in human depression (cortisol) and animal model (corticosterone) [11]. Because the function of glucocorticoid is mediated by glucocorticoid receptor (GR), current ideas support that the GR participates in the mechanism of HPA axis dysfunction and depression. Clinical evidence has shown that the hippocampus of depressed patients exhibits down-regulation of the GR expression (mRNA and protein) level which in turn leads to an increase in glucocorticoid [6,12,13]. Consistently, much strong evidence indicated that antidepressants can attenuate the decreases of hippocampal GR mRNA and protein expression [14–16].

Besides GR, brain-derived neurotrophic factor (BDNF), one of the most extensive neurotrophins, has been suggested to be involved in the pathomechanism of depression [17]. BDNF levels are reduced in the postmortem brains of depressed patients as well as in the animal models of depression [18,19]. In addition, a growing number of clinical and experimental evidence reports that the alterations in BDNF levels are associated with the beneficial therapeutic activities of antidepressant drugs [18,20]. Hence, BDNF has been considered as a possible target for antidepressants.

Emodin, 1,3,8-trihydroxy-6-methylanthraquinone (Fig. 1), the major active compound of Rhubarb, has been shown to have anti-cancer, liver protection, anti-inflammation, antioxidant and anti-virus effects [21]. Recently, it has been reported that emodin and its derivative has neuroprotective effects in severe cerebral injury [22,23]. Some previous research suggests that emodin could protect the brain from glutamate excitotoxicity via decreasing the release of glutamate [24]. And, there is evidence that emodin inhibits lipid peroxidation in rat brain homogenates [25].

Based on the above premises, the target of our research was to investigate whether oral emodin treatments at doses of 20 mg/kg, 40 mg/kg and 80 mg/kg possess potential antidepressant-like effects on chronic stress depression mouse model. Behavioral tests were performed to evaluate whether emodin could oppose CUMS-induced depression in mice. Meanwhile, we analyzed plasma corticosterone concentration and detected hippocampal GR and BDNF levels to elucidate the possible molecular basis of its antidepressant effects.

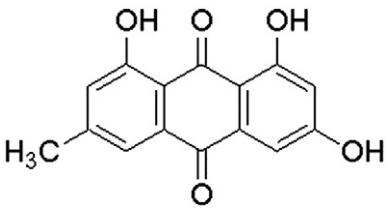

Fig. 1. The construction of emodin.

2. Materials and methods

2.1. Animals

Male ICR mice weighing 18–22 g (same batch) were purchased from Experimental Animal Center in Jiangsu Province (Nanjing, China). Prior to any experimentation, the mice were allowed to have one week to acclimatize to the laboratories. And, during the whole study, the mice were housed in group in a constant laboratory conditions at a temperature of 22 °C and 60% relative humidity under a 12 h light/12 h dark cycle. In our study, all the experimental procedures and laboratory animal care were performed in accordance with the National Institutes of Health (NIH) Guide.

2.2. Drug and treatment

Emodin was purchased from Nanjing Jiancheng Co., Ltd. Fluoxetine hydrochloride (positive control drug) was obtained from Changzhou Siyao Pharmaceuticals Co., Ltd (Changzhou, PR China). Emodin and fluoxetine were dissolved in 0.03% sodium carboxymethyl cellulose (CMC-Na). Mice were randomly divided into six different groups: one control group; one CUMS-vehicle (0.03% CMC-Na) group; one CUMS-FLU (20 mg/kg) group; and three CUMS-Emodin (20, 40, 80 mg/kg) groups. Each group included 10–12 mice (control: $n = 12$; CUMS + vehicle: $n = 10$; CUMS + FLU: $n = 11$; CUMS + Emodin 20 mg/kg: $n = 10$; CUMS + Emodin 40 mg/kg: $n = 11$; CUMS + Emodin 80 mg/kg: $n = 12$). Every morning, all drug treatment groups were administered orally via intragastric gavage in a dose of 10 ml/kg body weight.

2.3. Chronic unpredictable mild stress (CUMS) procedures

Table 1 shows the CUMS procedure which was performed according to the described protocol [26–28] with slight modifications. Briefly, the stressed groups were exposed to a variety of unpredictable stressors; (1) food deprivation (24 h), (2) water deprivation (24 h), (3) overnight illumination, (4) cage tilt (45°), (5) soiled cage (200 ml water in 100 g sawdust bedding), (6) exposure to a foreign object, (7) light/dark perversion, (8) overhang (10 min), (9) exposure to a empty bottle, (10) 1-min tail pinch (1 cm from the beginning of the tail), (11) 5 min oscillation, and (12) white noise. Animals of control group were undisturbed except for necessary housekeeping procedures.

The whole experiment lasted 42 days, and the procedural sequence was as follows: (1) stress procedure: days 1–42; (2) drug administration: days 21–42; (3) sucrose consumption test: 0-week, 3-week, and 6-week; (4) tail suspension