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Etazolate, a phosphodiesterase 4 inhibitor reverses chronic unpredictable mild stress-induced depression-like behavior and brain oxidative damage

Ankur Jindal *, Radhakrishnan Mahesh, Shvetank Bhatt

Department of Pharmacy, Birla Institute of Technology & Science, Pilani 333031, Rajasthan, India

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ABSTRACT

Etazolate, a pyrazolopyridine class compound is selective inhibitor of type 4 phosphodiesterase (PDE4). Previous study in our laboratory has demonstrated that etazolate produced antidepressant-like effect in rodent models of behavioral despair. The present study was designed to investigate whether etazolate could affect the chronic unpredictable mild stress (CUMS)-induced depression in mice. The effect of etazolate on CUMS-induced depression was examined by measuring behavioral parameters and oxidant/antioxidant status of brain tissue. Mice were subjected to different stress paradigms daily for a period of 28 days to induce depressive-like behavior. The results showed that CUMS caused depression-like behavior in mice, as indicated by significant ($p<0.05$) decrease in sucrose consumption and increase in duration of immobility. Moreover, CUMS also significantly ($p<0.05$) increased the oxidative stress markers and decreased the antioxidant enzymes activity. Chronic administration of etazolate (0.5 and 1 mg/kg., p.o.) and fluoxetine (20 mg/kg., p.o.) significantly ($p<0.05$) inhibited the CUMS-induced behavioral (decreased sucrose consumption and increased duration of immobility) and biochemical (increased lipid peroxidation and nitrite level; decreased glutathione, superoxide dismutase and catalase activity) changes. No alteration was observed in locomotor activity. Additionally, in the present study, the efficacy of etazolate (1 mg/kg., p.o.) on the behavioral and biochemical paradigms was found comparable to that of fluoxetine, used as standard antidepressant. In conclusion, the results of the present study suggested that etazolate alleviated the CUMS-induced depression in mice, which is at least in part mediated by modulating oxidative–nitrosative stress status in mice brain.

1. Introduction

Depression is a common incapacitating and life threatening psychiatric disorder which affects up to 21% population across the globally and associated with significant morbidity and mortality (McKenna et al., 2005; Nemeroff, 2007; Maes et al., 2009). According to World Health Organization prediction, it will be the second leading contributor to common disease by the year 2020, illustrating the severity and impact of the depression disorder (Aan het Rot et al., 2009). As per DSM-IV criteria, depression is characterized by depressed mood, reduced interest in usual activities and diminished ability to experience pleasure (American Psychiatric Association, 1994; Hankin, 2006). The etiological and pathological mechanisms underlying this disorder are not yet well understood, however, a growing body of clinical data suggests that stressful experiences play an important role in the onset and relapse of depression in humans (Lee et al., 2002; Charney and Manji, 2004; De Kloet et al., 2005; Heim et al., 2008).

Chronic unpredictable mild stress (CUMS) model of depression is widely used in preclinical antidepressants screening for investigating the pathophysiology of depression and the associated therapeutic interventions (Katz et al., 1981; Garcia et al., 2009). This model was developed in an attempt to resemble a variety of behavioral, neurochemical, neuroendocrine and neuroimmune alterations similar to those observed in human depressive disorder (Holsboer, 2000; McEwen, 2005). Moreover, it is suggested that chronic stress induces the oxidative damage in the central nervous system (CNS), which may be a possible mechanism in the pathophysiology of depression disorder (Moretti et al., 2012).

In fact, one of the attempts to explain the etiology of the depression disorder is the hypothesis of oxidative stress (Michel et al., 2007). Previous studies reported that repeated and unpredictable stress has a significant impact on reactive oxygen species (ROS) formation in brain, which in turn results in oxidative damage in the CNS (Madrigal et al., 2001; Fontella et al., 2005; Lucca et al., 2009). Induction of high oxidative stress in brain is considered as a major factor for neurotoxicity towards the pathophysiology of chronic stress-induced depression disorder (Tsuboi et al., 2006; Berk, 2007; Sarandol et al., 2007; Rothman and Mattson, 2010).

Etazolate (1-Ethyl-4-[(1-methylethylidene) hydrazino]-1H-pyrazolo-[3,4-b]-pyridine-5-carboxylic acid, ethyl ester hydrochloride) is a selective inhibitor of PDE4 enzyme (Wang et al., 1997; Drott et al., 2010; Jindal et al., 2012). Considerable research has been shown that

* Corresponding author. Tel.: +91 8890657646(mobile); fax: +91 1596 244183.
E-mail address: kumarjindal26@gmail.com (A. Jindal).

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inhibition of PDE4 enzyme increases intracellular availability of cAMP and influences the cAMP/PKA/CREB signaling cascade (Manji and Duman, 2001). cAMP/PKA/CREB signaling cascade implicates in the regulation of synaptic plasticity and neuronal survival in depression disorder (Shelton et al., 1996; Silvestre et al., 1999; Manji and Duman, 2001). Several studies reported that PDE4 inhibitors stimulate the cAMP/CREB signaling cascade by decreasing oxidative stress in brain (MacKenzie and Houslay, 2000). In addition, Sharma et al. (2012) have been investigated the role of PDE4 inhibitor in attenuation of oxidative stress and restoration of the antioxidant mechanism in brain.

Clinical studies have been reported that etazolate is a well tolerated drug with less side effects and currently receiving significant consideration among the clinical neurosciences (Wang et al., 1997; Drott et al., 2010). Earlier, preclinical studies have demonstrated the anxiolytic-like effect of etazolate (Beer et al., 1972; Horovitz et al., 1972). Recently, for the first time our group has demonstrated that treatment with etazolate produced antidepressant-like effects in rodent models of behavioral despair (Jindal et al., 2012). However, the effect of etazolate on CUMS-induced depression like behavior is still unknown and needs to be explored to identify its potential usefulness in the treatment or prevention of depression disorder. In addition, we also investigated the underlying mechanism of etazolate in CUMS, particularly with reference to oxidative damage in depression disorder. To the best of our knowledge, no study has as yet investigated the role of etazolate on the behavioral paradigms and oxidative damage in CUMS models of depression. Therefore, the present study was designed to investigate the effect of etazolate on CUMS-induced depressive behaviors and brain oxidative stress.

2. Materials and methods

2.1. Experimental animals

Behavioral experiments were carried out using Swiss Albino mice (22–25 g), procured from Hissar Agricultural University, Haryana, India. The animals were maintained in standard laboratory conditions (temperature 22 ± 2 °C and room humidity, 60 ± 10%) with a 12:12 h light/dark cycle. The animals were fed with standard diet and filtered water ad libitum. The experimental procedures on animals were in compliance with the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/14/03).

2.2. Schedule for drug administration and behavioral tests

Etazolate hydrochloride was purchased from the Tocris bioscience, UK. Fluoxetine was kindly provided by Glenmark Pharmaceuticals Ltd, India as generous gift sample and used as positive control for antidepressant action. All other biochemical reagents and solvents used in the study were of analytical grade. Etazolate and fluoxetine were dissolved in distilled water and always freshly prepared before administration. The drugs were administered by oral gavage (p.o.) once a day during the last 21 days (8th –28th days) of the CUMS procedure and continued till 31st day of the study. The behavioral testing was done at least 16–18 h after the last dose in order to avoid the acute effect of drug treatment. As shown in Fig. 1 behavioral tests of depression were performed during three days (29th–31st). The doses of etazolate and fluoxetine were selected according to the previous studies (Mao et al., 2009; Jindal et al., 2012).

2.3. Experimental design

Forty mice (eight mice in each group) were randomly divided into five different groups. Group I consisted of control unstressed mice; group II comprised of mice subjected to a series of different types of stressors for 28 days; group III and IV consisted of stressed mice received etazolate (0.5 and 1 mg/kg, p.o.) and group V consisted of stressed mice received fluoxetine (20 mg/kg, p.o.).

2.4. Chronic unpredictable mild stress procedure

Exposure to a single severe or repetitive, uncontrollable stressor may trigger or facilitate the development of psychopathologies. The CUMS procedure was performed as described by Ducottet et al. (2003), with slight modifications. This animal model of stress consists of chronic exposure to variable unpredictable stressors, none of which is sufficient alone to induce long-lasting effects. Briefly, CUMS consisted of exposure to a variety of unpredictable stressors (randomly); (1) food deprivation, (2) water deprivation, (3) exposure to a empty bottle, (4) cage tilt (45°), (5) overnight illumination (60-W lamp), (6) soiled cage (200 ml water in 100 g sawdust bedding), (7) forced swimming at 12 °C, (8) physically restraint (placing the animal in a plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move) and (9) exposure to a foreign object (e.g., a piece of plastic). These stressors were randomly scheduled over a 1 week period and repeated throughout the 4 weeks experiment

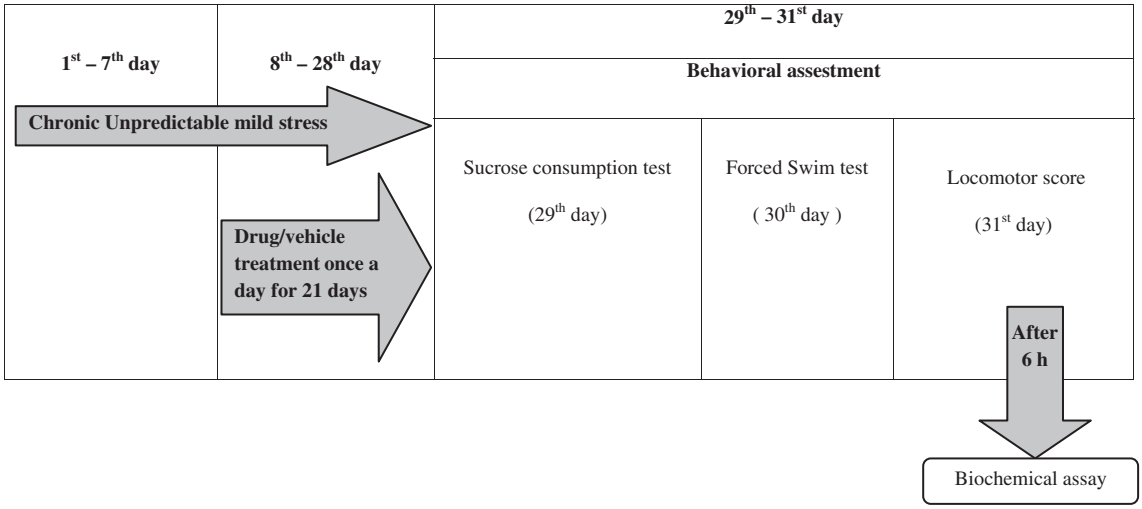

Fig. 1. Schematic representation of the experimental protocol indicating both the treatment schedule and the time of behavioral testing. Mice were sacrificed by decapitation 6 h after completion of behavioral testing on day 31, blood samples were collected and brain was dissected and stored at −80 °C for subsequent biochemical analysis.