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Original research article

Augmentation of effect of venlafaxine by folic acid in behavioral paradigms of depression in mice: Evidence of serotonergic and pro-inflammatory cytokine pathways

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ABSTRACT

Background: Though venlafaxine is an antidepressant with similar efficacy as selective serotonin receptor inhibitors, dose dependent adverse effects limit its use. Depression is associated with increased levels of pro-inflammatory cytokines.

Methods: The study investigated the effect of combining low/serotonergic dose of venlafaxine with folic acid in mice exposed to chronic forced swim stress for 21 days during which immobility and swimming time following forced swim test (FST) and immobility time in tail suspension test (TST) were measured every 7th, 14th and 21st day. The serum level of pro-inflammatory cytokines (IL-1β and IL-6) and whole brain levels of monoamines (serotonin, norepinephrine and dopamine) were estimated.

Results: An augmentation of antidepressant effect was observed in both forced swim test and tail suspension test following combination of venlafaxine (2 and 4 mg/kg) with folic acid (5 and 10 mg/kg) after 14 and 21 days of treatment. On brain serotonin level also, a significant augmentation was observed when venlafaxine (4 mg/kg) was combined with folic acid (10 mg/kg). Further, the combination significantly reversed the elevated levels of serum pro-inflammatory cytokines, IL-1β and IL-6 observed in chronic FST-induced stressed mice.

Conclusions: Combining low dose venlafaxine with an augmenting agent like folic acid, thus, appears to be an optimum strategy to increase its therapeutic efficacy and to reduce its dose.

Introduction

Major depressive disorder (MDD) is a chronic disorder that can impair a patient's regular day-to-day activities as well as social interactions [1,2]. WHO ranks depression as the 4th leading cause of disability worldwide and it is expected that by 2020, it will emerge as the second leading cause [3,4]. The first line treatment for the condition is antidepressant mono-therapy. But, almost 2/3rd of patients fail to achieve remission [5,6]. The combination therapy is considered to have additive/synergistic effects [7–9]. It has many advantages like rapid onset, comparable onset rates, therapeutic effects maintenance, avoid withdrawal symptoms of first line drugs and reduce adverse effects [10].

Venlafaxine is an effective serotonin norepinephrine reuptake inhibitor (SNRI) class of antidepressant. Higher rates of discontinuation due to adverse effects are considered to be the major drawback of venlafaxine [11]. Some of the common adverse effects associated with venlafaxine use are chills, hypertension, anorexia, nausea, agitation, dizziness, somnolence, tremor, yawning, sweating and abnormal ejaculation. Most of these adverse effects are dose dependent [12]. So, combining low dose venlafaxine with some other adjuvant agents appears to be an optimum strategy.

Folic acid is a water soluble B-vitamin essential for the synthesis of purine and thymidine nucleotides and for the synthesis of methionine from homocysteine [13,14]. Hyperhomocysteinemia, an indication of folate deficiency, is associated with poorer response to antidepressant medication which is improved by folate supplementation. Thus, folate has been proposed to have therapeutic potential as augmentation strategy in MDD [15,16]. Recently, folic acid has been reported to have augmenting action, when combined with fluoxetine [17].

MDD has been associated with increased levels of pro-inflammatory cytokines including TNF-α, IL-6 and IL-1β [18–20]. Cytokine hypothesis suggests depression as a consequence of immune

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activation followed by a state of immunosuppression [21]. Clinical studies suggest that inflammatory cytokines can be lowered in serotonergic (low) dose of venlafaxine [22]. Venlafaxine may restore the equilibrium between the physiological and pathological levels of cytokine secretion in depressive patients, leading to recovery [23]. There has been evidence of modulation of cytokines by folic acid. While it reduces IL-1β induced gingival tissues in patients using phenytoin and their effects on IL-1β in type I diabetes is under investigation [24]. In the above context, we combined venlafaxine with folic acid in stress-induced models of depression focusing on the inflammatory pathway. Although effects of venlafaxine and folic acid *per se* on experimental behavioral models of depression including forced swimming test (FST) and tail suspension test (TST) has been reported previously [25,26], their combinations have never been tested in animal models.

Materials and methods

Animals

Swiss albino mice weighing 30–40 g bred in Central Animal House facility, Jamia Hamdard, New Delhi were used. The animals were housed under a 12-h/12-h light and dark cycle and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiments. All the experiments were carried out between 09.00 h and 17.00 h. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) (742/CPCSEA, 2011) and conducted as per the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi. Each group comprised of eight animals.

Drugs, doses and treatment schedule

The drugs used were venlafaxine (Ranbaxy Laboratories Ltd, Gurgaon, India) and folic acid (Microlabs Ltd, Banglore, India). Venlafaxine was dissolved in normal saline and was given by intraperitoneal (*ip*) route at doses of 2–16 mg/kg, while folic acid was dissolved in drinking water and given by oral (*po*) route at doses of 5–50 mg/kg. These doses were selected by referring the previous literature on preclinical studies [26]. Lower doses of venlafaxine (2–4 mg/kg) were combined with lower doses of folic acid (5–10 mg/kg). To test the hypothesis that the anti-depressant effect of venlafaxine is augmented by folic acid, animals were treated with folic acid, half an hour before venlafaxine treatment.

Evaluation for antidepressant activity was performed on 7th, 14th and 21st days by chronic FST and TST. After the behavioral tests on day 21, blood was collected from the tail vein of animals, serum separated and tested for levels of serum IL-1β and IL-6 using mouse ELISA kits. Brain monoamine levels were evaluated by liquid chromatography mass spectroscopy (LCMS) and high performance liquid chromatography (HPLC) methods [27,28].

Behavioral tests

FST and TST, the widely accepted behavioral paradigms were used for assessing the antidepressant efficacy.

Forced swimming induced stress (chronic FST)

The animals were forced to swim individually in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25 ± 1 °C [29,30]. After an initial period of vigorous activity, each animal assumed a typical immobile posture. They were forced to swim for a 6 min session each day for 21 days to induce

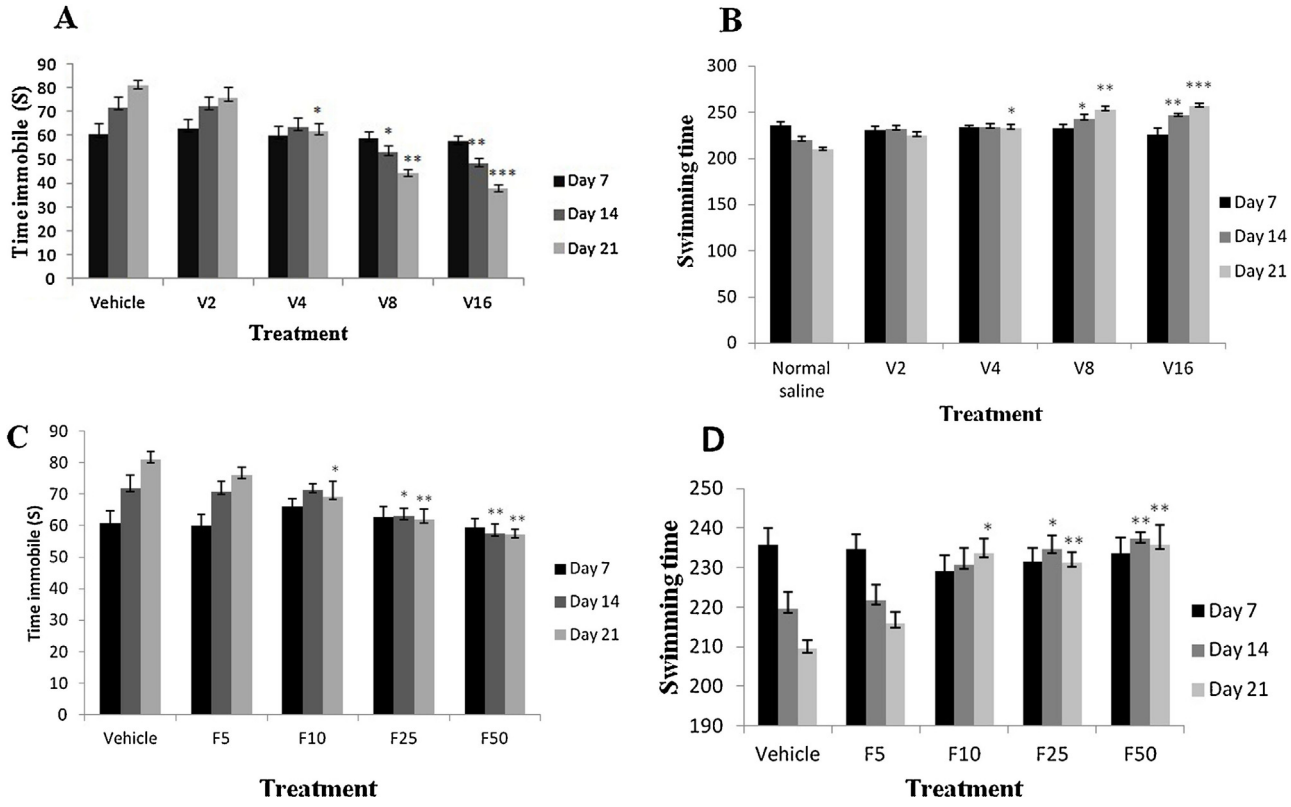

Fig. 1. Effects of various doses of venlafaxine (A, B) and folic acid (C, D) on immobility time and swimming time of chronic forced swim test on 7th, 14th and 21st days. Data are represented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to vehicle control group (one-way ANOVA followed by Tukey's test). V_2/venlafaxine 2 mg/kg, V_4/venlafaxine 4 mg/kg, V_8/venlafaxine 8 mg/kg, V_{16}/venlafaxine 16 mg/kg, F_5/folic acid 5 mg/kg, F_{10}/folic acid 10 mg/kg, F_{25}/folic acid 25 mg/kg, F_{50}/folic acid 50 mg/kg.