Pharmacological Reports 69 (2017) 143–149

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Adjuvant neuronal nitric oxide synthase inhibition for combined treatment of epilepsy and comorbid depression

Tanveer Singh, Rajesh Kumar Goel*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, Punjab, India

ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form 30 September 2016
Accepted 3 October 2016
Available online 4 October 2016

Keywords:
7-Nitroindazole
Corticosterone
Depression
Epilepsy
Nitric oxide
Pentylenetetrazole

ABSTRACT

Background: Elevated nitric oxide (NO) levels in the brain have been apparently associated with depression in kindled animals. Owing to the major role of neuronal nitric oxide synthase (nNOS) in brain and ineffectiveness of antiepileptic drugs (AEDs) in restoring nitrosative stress, the present study was envisaged to evaluate the adjuvant nNOS inhibitor, 7-nitroindazole (7-NI) with valproic acid for combined treatment of epilepsy and associated depression.

Methods: Pentylenetetrazole kindled animals associated with depression were treated with vehicle, valproate (300 mg/kg/day *ip*), valproate with 7-NI (10 mg/kg; 20 mg/kg; 40 mg/kg)/day *ip* and 7-NI (40 mg/kg/day *ip*) for 15 days. Except naïve, all groups were challenged with pentylenetetrazole (35 mg/kg *ip*) on days 5, 10, and 15 to evaluate seizure severity. Depression was evaluated in all experimental groups using the tail suspension and forced swim test on days 1, 5, 10 and 15. On day 15, biochemical (corticosterone levels) and neurochemical (serotonin, kynurenine, tryptophan, glutamate, GABA, nitrite levels) estimations were carried out in cortical and hippocampal area of mice brain.

Results: Vehicle treated kindled animals were significantly associated with depression. Chronic valproate treatment in kindled animals significantly reduced seizure severity, but could not reverse associated depression. 7-NI *per se* treatment in kindled animals was also reported unable to restore the associated depression completely. However, 7-NI supplementation with valproate significantly reduced seizure severity score and completely ameliorated depression with restoration of altered biochemical and neurochemical milieu.

Conclusion: Adjuvant nNOS inhibition can be previewed as safe therapy with AEDs for the combined management of epilepsy and associated depression.

Introduction

Epilepsy is a neurological disorder characterized by predisposition to generate seizures complicated with comorbid depression [1]. The comorbid depression has been found common in about 50% of patients with temporal-lobe epilepsy [2]. Although depression has characteristically intellectualized as a complication of the epilepsy, but it has also been reported otherwise too, as epidemiologic data underline that people with depression have a 7–fold increased risk of developing epilepsy [3]. The other major concern regarding this issue is that apart from epilepsy itself, the administration of antiepileptic drugs (AEDs) further worsens the comorbid depression [2].

Adding to the woes, the treatment of comorbid depression with available antidepressants (ADs) lowers the seizure threshold. The ADs such as imipramine, bupropion, maprotiline, clomipramine and amoxapine were reported to be the most notorious to aggravate seizures in people with epilepsy (PWE) [2]. The clinical data associated with the use of new generation ADs such as selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs) are also controversial with regard to their safety and efficacy in PWE [4]. Recently, vilazodone has also been reported with breakthrough seizures when used for treatment of depression in a patient with a history of epilepsy [5]. The inconclusiveness of available ADs regarding their safety and efficacy for the treatment of comorbid depression has also been endorsed by preclinical reports [6]. Thus, the current situation forbids the use of ADs and search of novel therapies for management of depression associated with epilepsy. National Institute of Neurological Disorders and Stroke (NINDS) epilepsy research benchmark III emphasized the prominence of comorbid

* Corresponding author.
E-mail address: goelrkpup@gmail.com (R.K. Goel).

http://dx.doi.org/10.1016/j.pharep.2016.10.001

depression and warrants safe approaches for the management of depression in epilepsy [7].

Our preliminary neurochemical study evidenced elevated nitric oxide (NO) levels in both cortical and hippocampal areas of the mice brain in pentylenetetrazole kindled animals [8]. The pentylenetetrazole kindling activates the glutamatergic pathway *via* the NMDA receptors, and then NO is generated by NMDA receptor-coupled neuronal nitric oxide synthase (nNOS) [9–11]. The elevated nNOS levels have been reported to mediate the loss of blood brain barrier integrity, one of the major pathological reasons for disrupting brain circuitry and neurochemical milieu thus accelerating epileptogenesis [9,11,12]. The pathogenic role of NO in epilepsy has been ambiguous as suggested by the controversial anticonvulsant effects of both NO enhancers as well as NO scavengers (NOS inhibitors) [13]. However, other studies have suggested elevated NO levels as consequence of seizures acting probably as an adaptive response to the sustained release of excitatory amino acids [9,11].

Whereas the elevated nNOS levels leading to enhance NO synthesis, have been unanimously convicted for depression [14]. Hippocampal nNOS mediated elevated NO levels may induce depressive behavior by downregulating glucocorticoid receptors (GRs) and decreasing serotonin signaling in the dorsal hippocampal area of the brain [15–17]. The antidepressant potential of various nNOS inhibitors indirectly suggests nNOS mediated elevated NO levels to be responsible for depression [14]. The inability of most of the AEDs to curb elevated NO levels support ameliorative role of adjuvant nNOS inhibition for treatment of depression in epilepsy [8,18]. nNOS inhibition has been reported not to interfere with the anticonvulsant effect of various AEDs [13,19,20]. The IDO inhibitory potential of nNOS inhibitors may further enhance antidepressant potential of nNOS inhibitors [21]. However, the efficacy and safety of nNOS inhibitors as an antidepressant has never been explored for the treatment of comorbid depression in epilepsy. Thus, an attempt has been made to evaluate the efficacy and safety of adjuvant nNOS inhibition with valproic acid for combined treatment of epilepsy and comorbid depression in pentylenetetrazole kindled animals.

Material and methods

Drugs and chemicals

All chemicals used in this study were of analytical grade. Pentylenetetrazole, kynurenine, tryptophan, serotonin, 7-nitroindazole (7-NI) were procured from Sigma-Aldrich, Co., St. Louis, MO, USA. GABA was acquired from Central Drug House (CDH, New Delhi, India); glutamate from S.D Fine Chem., Mumbai; HPLC-grade methanol and heptane sulfonic acid was procured from Merck Specialties (Mumbai, India). Perchloric acid was purchased from Spectrochem (Mumbai, India). 7-NI was suspended in 5% Tween 80 solution for intraperitoneal injection.

Animals

This study was carried out in two months aged male Swiss albino mice weighing 22–28 g. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (IAEC).

Kindling induction

Pentylenetetrazole was used for inducing kindling in mice employing the method previously validated [8]. Briefly, pentylenetetrazole dissolved in normal saline was injected intraperitoneally at sub-convulsive dose of 35 mg/kg at 48 ± 2 h intervals. After each injection, the mice were placed individually into plexiglass cages (20 × 20 × 30 cm) and observed for 30 min. The intensity of the convulsions was observed according to Racine's scale [8]. The animals were considered kindled after five times appearance of stage 5 (tonic–clonic convulsions) seizures on consecutive pentylenetetrazole administrations.

Experimental protocol

Total 48 animals were employed in this study. The group I: NAIVE, (non-kindled, n = 6) treated with saline and rest 42 animals were subjected to pentylenetetrazole induced kindling. The kindled animals were employed in the study and randomly divided into group II to VII. Group II: VHE, vehicle treated kindled animals (n = 6); Group III: VAL, valproate (300 mg/kg/day *ip*) treated kindled animals (n = 6); Group IV–VI: N10, N20, N40, valproate (300 mg/kg/day *ip*) with 10, 20, 40 mg/kg/day *ip*. 7-NI dose (n = 6 each); Group VII: N40 *per se*, 7-NI (40 mg/kg/day *ip*) (n = 6). All the groups except naïve were given chronic treatment for 15 days. The dose of 7-NI was selected in a dose range shown to inhibit nNOS as well as having no significant effect on cardiovascular system [22]. On every 5th day during treatment, groups II–VII was challenged with a subconvulsant pentylenetetrazole dose (35 mg/kg; *ip*) 45 min after the respective treatments, and the seizure severity score was registered. Animals were monitored for the seizure severity score up to 1 h (immediately after pentylenetetrazole subconvulsive dose). 2 h after the pentylenetetrazole subconvulsive dose once their locomotor activity becomes normalized, the animals were subjected to behavioral evaluations. On the 15th day, 4 h after behavioral assessment (for excluding any potential effect of behavioral

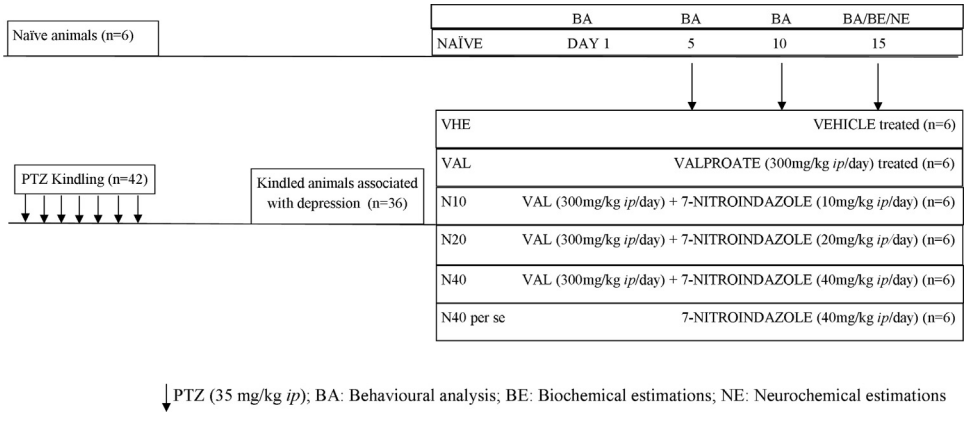

Fig. 1. Schematic representation of experimental protocol.