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Nitric oxide mediates the anticonvulsant effects of thalidomide on pentylenetetrazole-induced clonic seizures in mice

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ABSTRACT

Thalidomide is an old glutamic acid derivative which was initially used as a sedative medication but withdrawn from the market due to the high incidence of teratogenicity. Recently, it has reemerged because of its potential for counteracting number of diseases, including neurodegenerative disorders.

Other than the antiemetic and hypnotic aspects, thalidomide exerts some anticonvulsant properties in experimental settings. However, the underlying mechanisms of thalidomide actions are not fully realized yet. Some investigations revealed that thalidomide could elicit immunomodulatory or neuromodulatory properties by affecting different targets, including cytokines (such as TNF α), neurotransmitters, and nitric oxide (NO). In this regard, we used a model of clonic seizure induced by pentylenetetrazole (PTZ) in male NMRI mice to investigate whether the anticonvulsant effect of thalidomide is affected through modulation of the L-arginine–nitric oxide pathway or not.

Injection of a single effective dose of thalidomide (10 mg/kg, i.p. or higher) significantly increased the seizure threshold ($P < 0.05$). On the one hand, pretreatment with low and per se noneffective dose of L-arginine [NO precursor] (10, 30 and 60 mg/kg) prevented the anticonvulsant effect of thalidomide. On the other hand, NOS inhibitors [L-NAME and 7-NI] augmented the anticonvulsant effect of a subeffective dose of thalidomide (1 and 5 mg/kg, i.p.) at relatively low doses. Meanwhile, several doses of aminoguanidine [an inducible NOS inhibitor] (20, 50 and 100 mg/kg) failed to alter the anticonvulsant effect of thalidomide significantly. In summary, our findings demonstrated that the L-arginine–nitric oxide pathway can be involved in the anticonvulsant properties of thalidomide, and the role of constitutive nNOS is prominent in the reported neuroprotective feature.

1. Introduction

Thalidomide was introduced as a sedative/tranquilizer/antiemetic drug more than 50 years ago [1]. Because of the high incidence of teratogenicity with thalidomide, it was withdrawn from markets as a nonbarbiturate hypnotic in the early 1960s [2]. However, this drug was reconsidered recently, especially as an immunomodulator for counteracting different inflammatory and noninflammatory conditions including malignant disease, multiple myeloma, and HIV [2,3].

Recently, thalidomide has shown some neuroprotective properties [4] such as improvement in epilepsy [5] and memory deficit [6], besides having immunomodulatory effects. It has been suggested that thalidomide exerts some of these effects through inhibition of tumor necrosis factor alpha (TNF α) [6]. Some current studies have investigated the anticonvulsant properties of thalidomide in humans and in murine models, but definite underlying mechanisms for these effects have not yet been established [5,7].

Thalidomide interacts with many neurotransmitters in the body, and such interactions are involved in many pharmacological aspects of thalidomide [3,4,8,9]. Among these neurotransmitters, nitric oxide (NO) has received a great amount of attention and seems to act as a mediator for a number of thalidomide's effects [9–12]. Recent evidence from experimental studies implies that inhibition of the NO–cGMP

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pathway by thalidomide might mediate its central and peripheral effects, including modulation of neuropathic pain [13] and angiogenesis [9]. In the experimental model of demyelinating inflammatory disease, thalidomide treatment is associated with significant reduction in serum levels of NO [14]. Furthermore, thalidomide shows inhibitory activity toward nitric oxide synthase (NOS), especially neuronal NOS, which can explain the multiple central pharmacological effects elicited by this drug [15].

Nitric oxide is a soluble gaseous free radical, which is produced from the amino acid L-arginine by three different nitric oxide synthase (NOS) isoenzymes [16]. It functions as a neuronal messenger and as a modulator of neurotransmitters in the brain [17,18]. In CNS, NO is synthesized by the neuronal NOS (nNOS), a Ca-dependent enzyme. On the other hand, inducible NOS (iNOS) is a Ca-independent enzyme, which is involved in various inflammatory and pathophysiological processes like ischemia and stroke [19]. It has been confirmed that NO could mediate pathophysiologic aspects of seizures and seems to be an end product of many excitatory pathways, leading to seizures [20]. The important role of NO in the modulation of seizure threshold raises the hypothesis that thalidomide may affect seizure susceptibility through NO-dependent mechanisms.

In the present study, we assessed the possible contribution of the NO pathway in the anticonvulsant action of thalidomide by employing a model of clonic seizures induced by pentylenetetrazole (PTZ) in mice. We further investigated whether the constitutive or inducible NOS isoenzyme is involved in this phenomenon.

2. Materials and methods

2.1. Chemicals

The following compounds were used in this study: pentylenetetrazole (PTZ) (Sigma, UK), L-arginine (L-ARG), N^GL-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI), and aminoguanidine (AG) (Sigma, St Louis, MO, USA). Thalidomide was synthesized based on the Chemie Grunenthal method [21]. Thalidomide, L-arginine, L-NAME, 7-NI, and AG were administered intraperitoneally in a volume of 10 ml/kg of the mouse's body weight. 7-Nitroindazole was suspended in a 1% aqueous solution of Tween 80, and thalidomide was suspended in a 1% aqueous solution of DMSO. All other drugs were dissolved in normal saline. To induce clonic seizures, PTZ was administered intravenously (0.5%, iv). The doses were chosen based on our previously published study [20,22] and pilot experiments. In experiments with sequential treatments, the interval between administration of NOS inhibitors or L-arginine and that of thalidomide was 15 min, so that an effective blockade of enzymes by inhibitors was allowed.

2.2. Subjects

Male NMRI mice weighing 26 ± 3 g (Razi Institute, Karadj, Iran) were used in the study. The animals were housed in standard polycarbonate cages in groups of 4–5 and kept in a temperature-controlled room (22 °C) with a 12-h light/12-h dark cycle. Animals were acclimated at least 2 days before experiments with free access to food and water. The experiments were conducted between 09:00 and 14:00. All procedures were carried out in accordance with institutional guidelines for animal care and use. The groups consisted of at least eight animals, and each animal was used only once. Additionally, efforts were made to reduce animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.3. Seizure paradigms

To determine the clonic seizure threshold, we inserted a 30-gauge dental needle into the lateral tail vein of mice. The needle was then secured to the tail by a narrow piece of adhesive tape. With the mouse moving freely, the PTZ solution (0.5%) was slowly infused into the tail vein at a constant rate of 1 ml/min, using an infusion pump (NE 1000, New Era Pump System, Inc.), which was connected to the dental needle by polyethylene tubing. Infusion was halted when general clonus (forelimb clonus followed by full clonus of the body) was observed. The minimal dose of PTZ (mg/kg of mice weight) needed to induce general clonus was recorded as an index of clonic seizure threshold [23–25]. In this regard, the seizure threshold is dependent on PTZ dose administered and time-related.

2.4. Experiments

In experiment 1, different doses of thalidomide (0.5, 1, 5, 10, 20, and 50 mg/kg) were injected 30 min prior to the determination of clonic seizure threshold induced by intravenous administration of PTZ solution. Control animals received the same volume of the vehicle (1% aqueous solution of DMSO) in all experiments. The doses and time point were chosen on the basis of pilot studies. In experiment 2, regarding assessment of the time course of thalidomide, effective thalidomide dose (20 mg/kg) was administered 15, 30, 45, or 60 min prior to PTZ in distinct groups of mice. The corresponding control group received vehicle at the same time point. Based on these two experiments, the subeffective doses of 1 and 5 mg/kg of thalidomide with a pretest injection interval of 30 min were used for subsequent experiments. In experiment 3, we examined the effects of the NO precursor, L-arginine, on an effective dose of thalidomide; mice received acute administration of L-arginine (10, 30, and 60 mg/kg) 15 min before thalidomide administration (10 mg/kg) and 45 min before PTZ administration.

In experiment 4, different doses of L-NAME (1 and 5 mg/kg) were acutely administered alone and 15 min before thalidomide administration (1 and 5 mg/kg) and 45 min before PTZ administration to examine the effect of these combinations on seizure threshold. In experiment 5, animals received acute intraperitoneal injections of 7-NI (15 and 30 mg/kg) as a neuronal NOS inhibitor alone or 15 min before subeffective doses of thalidomide (1 and 5 mg/kg). In experiment 6, different doses of AG (20, 50, and 100 mg/kg) were acutely administered alone or 15 min before thalidomide administration (5 mg/kg) and 45 min before PTZ administration. This experiment was done to find the role of iNOS in thalidomide effects on the seizure threshold.

2.5. Statistical analysis

Results of seizure thresholds are expressed as the mean ± S.E.M. of clonic seizure threshold in each experimental group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. In all experiments, a P-value < 0.05 was regarded as significant.

3. Results

3.1. Effect of different doses of thalidomide on seizure threshold and its time courses

Fig. 1a illustrates the effect of acute intraperitoneal administration of different doses of thalidomide (0.5, 1, 5, 10, 20, and 50 mg/kg, i.p.) on the PTZ-induced clonic seizure threshold. One-way ANOVA revealed a significant effect for thalidomide ($F(6, 51) = 6.482$, $P < 0.001$), and post hoc analysis showed a significant anticonvulsant effect for thalidomide at doses of 10 mg/kg and higher compared with vehicle-treated control animals. Fig. 1b shows the time course of the anticonvulsant properties for the effective dose of thalidomide (20 mg/kg, i.p.). Statistical analysis showed that thalidomide exerted a maximum anticonvulsant effect 30 min after administration ($P < 0.001$, compared with the vehicle-treated control group), and its effect decreased thereafter at 45 and 60 min after injection ($F(4, 32) = 5.538$, $P < 0.01$).