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Research article

The role of nitric oxide in anticonvulsant effect of nanocurcumine on pentylenetetrazole-induced seizure in mice

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HIGHLIGHTS

- Acute systemic nanocurcumin exerted dose-dependent anticonvulsant properties in PTZ-induced clonic seizure in mice.
- L-arginine as a NO donor prevented the anticonvulsant effects of nanocurcumin. L-NAME and AG potentiated the anticonvulsant effect of sub-effective nanocurcumin.
- NO overproduction probably from iNOS is involved on the seizure susceptibility and suppression of iNOS activity may be one of the possible mechanisms by which nanocurcumin exerts its antiseizure activity.

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ABSTRACT

A plant alkaloid obtained from *Curcuma longa*, curcumin possesses anti-oxidant and anti-inflammatory effects. Nanoformulations have been developed for preclinical studies which demonstrate enhanced therapeutic efficacy. Effect of acute intraperitoneal (i.p.) administration of curcumin C3 complex nanoparticles [1,5, 10, 20, 40, 80 mg/kg, (i.p.)] 75 min prior to PTZ, on clonic seizure thresholds induced by intravenous infusion of pentylenetetrazole (PTZ) 0.5% was investigated in comparison with curcumin (40 and 80 mg/kg, i.p.) in male mice. Moreover, to clarify the probable role of NO in the anticonvulsant property of nanocurcumin, non-effective doses of L-arginine (L-Arg), a NO donor; 7-nitroindazole, 7-NI, a preferential neuronal NO synthase inhibitor; L-NAME, a non-selective NO synthase inhibitor and aminoguanidine (AG), a selective inducible NO synthase inhibitor (iNOS), in combination with nanocurcumin (80 mg/kg, i.p.), 15–30 min before it were employed.

Results: While curcumin did not show any anticonvulsant effect, nanocurcumin revealed dose-dependent anticonvulsant property at the doses 20, 40 and 80 mg/kg, $P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively. L-Arg (30 and 60 mg/kg) dose-dependently reversed the anticonvulsant effect of the most effective nanocurcumin dose (80 mg/kg), $P < 0.01$ and $P < 0.001$, respectively. On the other hand, L-NAME (3 and 10 mg/kg, i.p.) markedly potentiated the sub effective dose of nanocurcumin (10 mg/kg), $P < 0.01$ and $P < 0.001$, respectively. Similarly, AG (50 and 100 mg/kg, i.p.) profoundly augmented the seizure thresholds of nanocurcumin (10 mg/kg), $P < 0.01$ and $P < 0.001$, respectively. In addition, 7-NI (10, 30 and 60 mg/kg, i.p.) failed to influence the responses.

Conclusion: These data may support excess of NO production following PTZ infusion probably resulting from iNOS source. Consequently, nanocurcumin probably down regulated NO. To conclude, nanocurcumin showed anticonvulsant effect. Furthermore, this effect was reversed following L-arginine as an external NO precursor. However, both the non-selective NOS inhibitor and selective iNOS inhibitor

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increased the thresholds. It is evident that nanocurcumin may influence the seizure thresholds at least in part through a decrease in NO.

1. Introduction

Epilepsy is a chronic neurological disease, characterized by spontaneous recurrent seizures (SRS) with prolonged epileptic discharge which affects 1% of the world's population [1,2].

Curcumin [diferuloylmethane; 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadien- 3, 5-dione] is a yellow crystalline compound and the active ingredient of turmeric. It is a plant alkaloid obtained from *Curcuma longa* (Zingiberaceae) [3]. Curcumin possesses anti-oxidant [4], anti-inflammatory [5], anti-microbial, wound healing [6] and hypoglycemic effects [7]. It has shown to inhibit the mammalian TOR (mTOR) pathway, a key regulator of cell growth and proliferation as well [8]. Nevertheless, a major limiting factor of curcumin is its low solubility in water (i.e. 0.0004 mg/ml at pH 7.3) and soluble curcumin molecules are extremely sensitive at the physiological pH [9]. Many preclinical and clinical studies in mice, rats and humans revealed low curcumin bioavailability [10,11]. Consequently, curcumin's potential for therapeutic translation has been hindered by its low oral bioavailability, poor aqueous solubility, rapid degradation, and restricting clinical applicability [12]. Encapsulation of curcumin in a nanoparticle platform is a conceivable and advantageous means enabling its delivery. In view of their small size and high surface-to-volume ratio, nanoparticles can pass through the skin barrier [12]. Nanotechnology is an emerging field that is potentially improves the bioavailability, to increase the plasma concentration, and to enhance the cellular permeability processes of curcumin. Liposomal curcumin nanoparticles have better permeability and stronger resistance to metabolic processes [13]. Curcumin nanoformulations have been developed for preclinical studies on cancer, inflammation, wound healing, and other conditions, which demonstrate enhanced therapeutic efficacy with nano- versus non-encapsulated curcumin [12,14]. For instance, dendrosomal curcumin had a chemoprotective effect on breast cancer metastasis [15].

Curcumin has recently been reported to have anticonvulsant effects in several animal models of epilepsy [16–18]. Nevertheless, oral curcumin treatment had no effect on chronic seizures in the electrical post-status epilepticus (SE) rat model, possibly because it did not reach the brain at adequate levels [19]. Curcumin has ameliorative effect on seizure severity, depression like behavior and memory impairment of pentylenetetrazole (PTZ)-kindled mice, possibly via central monoaminergic modulation and inhibitory effect on nitrosative stress and acetylcholinesterase activity [20].

Nitric oxide (NO) a highly reactive messenger molecule which is synthesized in some tissues consisting of the brain is regarded as neuronal messenger or neurotransmitter in the central nervous system [21,22]. Moreover, NO is a known modulator of seizure susceptibility with various anticonvulsant [23,24] and proconvulsant impacts [25,26]. Studies have shown that there is increased NO production in several parts of the brain within experimentally-induced seizures [27]. PTZ −induced seizures increased inducible NO synthase inhibitor (iNOS) activity in the hippocampus. Considering the fact that higher activity of iNOS is associated with excitotoxicity, NO formation is amplified through epileptic seizures [28]. Expression of iNOS in the brain stimulates glutamatergic pathway and causes hyperexcitability [29].

Several studies suggest that NO mediates curcumin effects [30,31]. It has been indicated that curcumin can alleviate the subacute stress response through the modulation of NO production in the hippocampus [30]. Other experiments have indicated that the NO pathway is involved in the amelioration effects of curcumin in ethanol-induced memory impairments [31]. Curcumin inhibited the selective iNOS induction in activated macrophages and the NO production by mouse peritoneal macrophages [32,33]. Curcumin inhibited iNOS induction in macrophages activated with lipopolysaccaride (LPS) and interferon-gamma (IFN-γ) [5]. Also, it has shown that two oral treatments of 0.5 ml of a 10-microM solution of curcumin (92 ng/g of body weight) reduced iNOS mRNA expression in the livers of LPS-injected mice [33].

Anticonvulsant effect of curcumin in some animal seizure models and the involvement of NO pathway in its antiepileptic action have already demonstrated. Notably, nanocurcumin formulations have never been evaluated in any epileptic disorders. By virtue of the mentioned literature, we hypotheses that nanocurcumin may exert improved impact and efficacy on seizure disorder compared to non-capsulated curcumin in PTZ-kindled mice. Further, it was investigated whether a NO signaling pathway is also involved in the anticonvulsant effect of nanocurcumin.

2. Methods and materials

2.1. Animals

Male NMRI albino mice (24–30 g) were used for performing this experiment. The animals were housed in temperature-controlled room (24 ± 1 °C) on a 12-h light: 12-h dark cycle with free access to food and water. All the procedures were carried out in as indicated in the criteria proposed by the Guide for the Care and Use of Laboratory Animals (NIH US publication, no. 23–86, revised 1985). The experimental and the control groups consisted of 8 and 6 mice, respectively.

2.2. Pharmacological chemicals

Nanocurcumin (curcumin C3 complex-loaded nanoparticles) and native curcumin were purchased from Exir Nano Sina Co., Tehran, Iran. L-NAME, 7-nitroindazole, aminoguanidine, L-arginine and pentylenetetrazole were purchased from Sigma Co.

2.3. Pentylenetetrazole (PTZ)-induced clonic seizures

The conscious mice were restrained (no anesthesia), a 30-gauge butterfly needle was inserted into the tail vein and fixed by a piece of paper tape. The animals were placed in the middle of a shallow container and allowed to move freely. All these steps take less than 10 s and normally no mortality was observed. The infusion pump was adjusted to deliver PTZ (0.5%) at a constant rate of 1 ml/min in all the experiments (NE 1000, New Era Pump System, Inc.) [34]. The infusion was discontinued immediately subsequent to forelimb clonus which normally followed by a full clonus of the body [35,36].

Finally, the seizure threshold was calculated using the following formula: Index of seizure threshold (mg/kg) = (PTZ concentration [mg/ml] x infusion rate [ml/s] × infusion duration[s] × 1000)/weight of mouse [g]. The minimal PTZ dose (mg/kg