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Short communication

Acute toxicity and anticonvulsant activity of liposomes containing nimodipine on pilocarpine-induced seizures in mice

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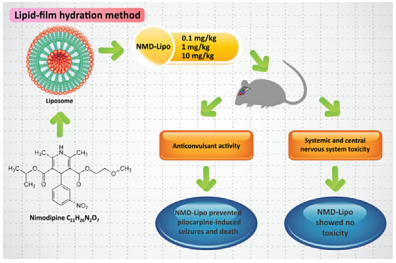
HIGHLIGHTS

- NMD-Lipo did not produce acute toxicity in mice.
- NMD-Lipo has anticonvulsant activity on pilocarpine-induced seizures in mice.
- NMD-Lipo showed anticonvulsant activity significantly major that free NMD.

GRAPHICAL ABSTRACT



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ABSTRACT

Nimodipine has been shown to have an inhibitory action on seizures and brain damage in rodents. However, the pharmaceutical applicability of this drug is limited by its low solubility in gastrointestinal fluids and high first-pass effect in the liver, which leads to low bioavailability. These difficulties can be overcome through the use of liposomes. The aim of the present study is to evaluate the toxicity and anticonvulsant activity of liposomes containing nimodipine (NMD-Lipo) on pilocarpine-induced seizures. NMD-Lipo was prepared using the lipid-film hydration method. Central nervous system toxicity of NMD-Lipo was assessed by Hippocratic screening. Systemic toxicity was evaluated by analyses of biochemical and hematological parameters and by observing possible signs of toxicity. The possible anticonvulsant activity was tested by the pilocarpine model. The administration of the NMD-Lipo at doses of 0.1, 1, and 10 mg/kg caused no toxicity in animals. Furthermore, NMD-Lipo prevented the installation of 100% of the pilocarpine-induced seizures and prevented the death of 100% of the mice treated with pilocarpine. These data shown that NMD-Lipo has an anticonvulsant activity significantly superior to free NMD, suggesting that the liposomes promoted a drug controlled release by improving its bioavailability and consequently increasing its pharmacological activity.

1. Introduction

Epilepsy is a chronic disease of the central nervous system characterized by recurrent seizures caused by excessive discharges of cerebral neurons. This condition is a health concern, as it is considered one of the most serious neurological disorders [1]. Clinically, patients with the disease experience a deterioration of one or more

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cognitive functions, with or without motor behavior and/or psychomotor decrease [2].

Seizures can be completely controlled with medical therapy in two-thirds of patients; however, one-third remains refractory to the medications [3]. Furthermore, the current antiepileptic drugs used in the treatment of epilepsy have a wide range of adverse reactions, toxicity, and teratogenic effects. Based on these findings, new therapeutic agents, which allow more efficient seizure control in resistant patients and with fewer side effects, are greatly needed [4].

Research has shown that the intrinsic epileptiform activity is associated with calcium (Ca^{2+}) influx through NMDA receptor-operated Ca^{2+} channels and through voltage-operated Ca^{2+} channels. Therefore, the inhibition of the intracellular Ca^{2+} increase represents an important target in the development of antiepileptic and neuroprotective drugs [5]. From this perspective, calcium channel blockers may be considered as a possible therapeutic agent for the disease.

Nimodipine (NMD) is a dihydropyridine L-type Ca^{2+} channel antagonist that crosses the blood–brain-barrier more easily than other calcium-channel-blockers and binds with high affinity and specificity to the calcium-channel receptors in the brain [6]. NMD has been shown to have an inhibitory action on seizures and brain damage in rodents [16–23]. However, the pharmaceutical applicability of nimodipine is limited by its low solubility in gastrointestinal fluids and high first-pass effect in the liver, which leads to low bioavailability after oral administration [7,8].

These difficulties can be overcome through the use of liposomes. These nanometer-scale pharmaceutical carriers are self-assembled colloidal vesicles consisting of one or more concentric phospholipid bilayers organized around an aqueous inner compartment, and are used to encapsulate drugs, biomolecules or diagnostic agents [9]. The aim of the present study is two fold: the evaluation of the nimodipine encapsulated into liposomes (NMD-Lipo) toxicity and the study of anticonvulsant activity of NMD-Lipo on pilocarpine-induced seizures.

2. Materials and methods

2.1. Reagents

Cholesterol (CHOL), trehalose, nimodipine, and pilocarpine hydrochloride were purchased from Sigma–Aldrich (St. Louis, USA). Soybean phosphatidylcholine (PC) (98% Epikuron 200) was obtained from Lipoid GMBH (Ludwigshafen, Germany). Solvents and other chemicals were supplied by Merck (Darmstadt, Germany).

2.2. Animals

Adult male Swiss mice (25–30 g; 2 months old) were obtained from Central Animal House of the Federal University of Piaui, Piaui, Brazil. They were maintained in a temperature controlled room ($25 \pm 1\,^{\circ}C$), with a 12 h light/dark cycle (lights on 07:00–19:00 h), and food and water provided *ad libitum* (Nutrilabor, Campinas, Brazil). The experimental protocols and procedures were approved by the Ethics Committee on Animal Experimentation of the Federal University of Piauí (CEEA/UFPI N° 014/11). All experiments were performed according to the guide for the care and use of laboratory of the US, Department of Health and Human Services, Washington, DC (1985).

2.3. Preparation and characterization of liposomes containing nimodipine

Liposomes containing nimodipine (NMD-Lipo) were prepared and characterized as previously described [10]. The content of nimodipine in liposomes was determined using UV spectroscopy at 237 nm and the encapsulation efficiency of nimodipine into liposomes was determined after the submission of samples to ultrafiltration/ultracentrifugation using Ultrafree® units (Millipore, USA), for the separation of the drug encapsulated and non encapsulated into liposomes [10]. The content of nimodipine in the supernatant was then determined and the drug encapsulation ratio was calculated as:

$$\%EE = \frac{[NMD]_{content} - [NMD]_{free}}{NMD_{content}} \times 100.$$

2.4. Systemic and central nervous system toxicity of NMD-Lipo

Mice were divided into four groups, with 16 animals in each group. The first group was treated with 0.9% saline. The second, third, and fourth groups were treated with NMD-Lipo at doses of 0.1, 1, and 10 mg/kg. NMD is a widely used drug and its security is well-known, so the toxicity tests have not been conducted with free NMD, only with NMD-Lipo.

Central nervous system toxicity of NMD-Lipo was assessed by Hippocratic screening. Systemic toxicity was evaluated by analysis of biochemical and hematological parameters and by observing possible signs of toxicity.

Half of the animals in each group ($n = 8$) were observed for 24 h and subsequently were intended to implement the blood tests. During this period we proceeded to the observation of the mice at the time of 30 min, 1, 2, 4, 8, 12, and 24 h for the purpose of quantifying the effect of NMD-Lipo on the following parameters: (a) state of awareness and readiness; (b) motor coordination; (c) muscle tone; (d) reflection (atrial and cornea); (e) central nervous system activity; (f) autonomic nervous system activity. At the end of 24 h, the animals were anesthetized with pentobarbital 40 mg/kg and blood was immediately collected from the retro-orbital plexus for the assessment of biochemical and hematologic parameters [11].

The other half ($n = 8$) was under observation for a period of 30 days for viewing and the recording of possible signs of toxicity of the formulation. During these 30 days, the consumption of water and feed was recorded daily, body weight of mice was measured every two days and the animals were evaluated for clinical signs of toxicity.

2.5. Anticonvulsant activity of NMD-Lipo

Mice were divided into twenty-two groups, with each group containing 12 animals. The negative control group was treated with 0.9% saline. The P400 group was treated with pilocarpine hydrochloride at a dose of 400 mg/kg to induce seizures. The third and fourth groups were treated with diazepam at a dose of 5 mg/kg and an association of diazepam with pilocarpine hydrochloride in a dose of 400 mg/kg. The fifth, sixth, and seventh groups were treated with empty liposomes at doses of 0.1, 1, and 10 mg/kg. The eighth, ninth, and tenth groups were treated with empty liposomes at doses of 0.1, 1, and 10 mg/kg and after 30 min they received pilocarpine hydrochloride at the dose of 400 mg/kg. The eleventh, twelfth, and thirteenth groups were treated with free nimodipine at doses of 0.1, 1, and 10 mg/kg. The fourteenth, fifteenth, and sixteenth groups were treated with free nimodipine at doses of 0.1, 1, and 10 mg/kg and after 30 min they received pilocarpine hydrochloride at the dose of 400 mg/kg. The seventeenth, eighteenth, and nineteenth groups were treated with NMD-Lipo at the doses of 0.1, 1, and 10 mg/kg. Finally, the animals of the twentieth, twenty-first, and twenty-second groups received NMD-Lipo at the doses of 0.1, 1, and 10 mg/kg and after 30 min they received pilocarpine hydrochloride at the dose of 400 mg/kg.