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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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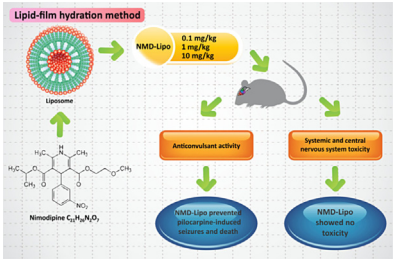
HIGHLIGHTS

- 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.

After the treatments, the animals were recorded in 30 cm × 30 cm chambers with: appearance of peripheral cholinergic signs (miosis, piloerection, chromodacryorrhea, diarrhea, and urination), stereotyped movements (continuous sniffing, paw licking, and rearing), tremors, seizures, status epilepticus, and mortality rate, during 24 h. We decided to observe possible changes in the behavior of mice for 24 h after pilocarpine administration because previous works showed that convulsions and deaths occurred within 1 and 24 h, respectively, post pilocarpine injection [12].

2.6. Statistical analyses

The results were presented as a percentage according to the number of animals used in the experiments. Peripheral cholinergic signs, stereotypic movements, tremor, seizures, status epilepticus, and mortality rate were presented as percentages and compared with a nonparametric test (Chi-Square test). In all situations statistical significance was reached at p less-than-or-equals, slant 0.05. The statistical analyses were performed with the software GraphPad Prism, version 6.00 for windows, GraphPad software (San Diego, CA, USA).

3. Results

3.1. Nimodipine-loaded liposomes

NMD-Lipo presented a drug content of 0.98 ± 0.58 mg/ml and encapsulation efficiency of 99 ± 0.22%.

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

In the Hippocratic screening, NMD-Lipo did not cause any behavioral alterations in mice at the doses tested. Therefore, no alteration was observed in biochemical and hematologic parameters as any variation in weight of mice treated with the formulation. None of the animals treated with NMD-Lipo died.

3.3. Behavioral alterations after pretreatment with NMD-Lipo

The results of the behavioral alterations of animals after pretreatment with NMD-Lipo after 24 h of phase acute of pilocarpine-induced seizures are summarized in Table 1. None of the mice that received injections of isotonic saline (negative control), diazepam, empty liposomes, free nimodipine, and NMD-Lipo unassociated with pilocarpine showed peripheral cholinergic signs,

Table 1
Effect of pretreatment with NMD-Lipo, free nimodipine, liposomes, and diazepam on pilocarpine-induced seizures and lethality in adult mice.

Groups (n = 12)	Peripheral cholinergic signs %	Stereotypic movements %	Tremor %	Seizures %	Status epilepticus %	Mortality rate %
Negative control		00	00	00	00	00
P400	100[a]	100[a]	100[a]	100[a]	75[a]	75[a]
DZP 5 plus P400	100[a]	100[a]	100[a]	50[a,b]	50[a,b]	50[a,b]
DZP 5	00	00	00	00	00	00
Lipo 0.1	00	00	00	00	00	00
Lipo 1	00	00	00	00	00	00
Lipo 10	00	00	00	00	00	00
NMD-Lipo 0.1 plus P400	100[a]	65[a,b,c,d,e]	100[a]	00[b,c,d,e]	00[b,c,d,e]	00[b,c,d,e]
Lipo 0.1 plus P400	100[a,c]	100[a,c]	100[a,c]	100[a,c]	75[a,c]	75[a,c]
Lipo 1 plus P400	100[a,d]	100[a,d]	100[a,d]	100[a,d]	75[a,d]	75[a,d]
Lipo 10 plus P400	100[a,e]	100[a,e]	100[a,e]	100[a,e]	75[a,e]	75[a,e]
Free NMD 0.1	00	00	00	00	00	00
Free NMD 1	00	00	00	00	00	00
Free NMD 10	00	00	00	00	00	00
Free NMD 0.1 plus P400	100[a,f]	100[a,f]	100[a,f]	100[a,f]	50[a,b,f]	75[a,f]
Free NMD 1 plus P400	100[a,g]	100[a,g]	100[a,g]	100[a,g]	50[a,b,g]	75[a,g]
Free NMD 10 plus P400	100[a,h]	75[a,b,h,*,**]	100[a,h]	100[a,h]	75[a,h,*,**]	75[a,h]
NMD-Lipo 0.1	00	00	00	00	00	00
NMD-Lipo 1	00	00	00	00	00	00
NMD-Lipo 10	00	00	00	00	00	00
NMD-Lipo 0.1 plus P400	100[a,i]	65[a,b,i,&]	100[a,i]	00[b,&]	00[b,&]	00[b,&]
NMD-Lipo 1 plus P400	100[a,j]	40[a,b,j,#,&]	60[a,b,j,#,##,&]	00[b,&]	00[b,&]	00[b,&]
NMD-Lipo 10 plus P400	100[a,l]	30[a,b,l,#,##,&]	20[a,b,l,#,##,&]	00[b,&]	00[b,&]	00[b,&]

Mice (25–30 g; 2 months old) were treated acutely with vehicle (saline 0.25 ml, negative control), pilocarpine (400 mg/k.g, i.p, P400), diazepam (5 mg/kg, i.p., DZP 5, positive control), empty liposomes (Lipo), free nimodipine (Free NMD), and liposomal formulation containing nimodipine (NMD-Lipo) at doses 0.1, 1 e 10 mg/kg (i.p.). Others groups of mice were pretreated acutely with Lipo, free NMD and NMD-Lipo at doses 0.1, 1 e 10 mg/kg (i.p.) or DZP and 30 min after treatment with pilocarpine 400 mg/kg, i.p. Results for peripheral cholinergic signs, stereotypic movements, tremor, seizures, status epilepticus, and death are expressed as percentages of the number of animals from each group.

[a] $p < 0.05$, when compared with negative control.
[b] $p < 0.05$, when compared with P400 group.
[c] $p < 0.05$, when compared with Lipo 0.1.
[d] $p < 0.05$, when compared with Lipo 1.
[e] $p < 0.05$, when compared with Lipo 10.
[f] $p < 0.05$, when compared with Free NMD 0.1.
[g] $p < 0.05$, when compared with Free NMD 1.
[h] $p < 0.05$, when compared with Free NMD 10.
[i] $p < 0.05$, when compared with NMD-Lipo 0.1.
[j] $p < 0.05$, when compared with NMD-Lipo 1.
[l] $p < 0.05$, when compared with NMD-Lipo 10.
[*] $p < 0.05$, when compared with Free NMD 0.1 plus P400.
[**] $p < 0.05$, when compared with Free NMD 1 plus P400.
[#] $p < 0.05$, when compared with NMD-Lipo 0.1 plus P400.
[##] $p < 0.05$, when compared with NMD-Lipo 1 plus P400.
[&] $p < 0.05$, when compared with DZP 5 plus P400 (Chi-Square test).

stereotypic movements, tremor, and seizures. None of the animals in these groups died.

All animals treated with P400 alone presented peripheral cholinergic signs and stereotyped movements followed by motor limbic seizures. The convulsive process persisted and built up to a status epilepticus in 75% of these mice, leading to death of 75% of the animals. Diazepam at the dose of 5 mg/kg did not significantly reduce the occurrence of peripheral cholinergic signs, stereotypic movements, and tremors. The benzodiazepine was able to reduce by 50% the occurrence of seizures and by 33.33% the mortality rate in mice. Empty liposomes at doses of 0.1, 1, and 10 mg/kg did not reduce the occurrence of peripheral cholinergic signs, stereotypic movements, and tremor. Liposomes without nimodipine were unable to prevent the installation of the seizure and decrease the mortality rate in rodent. Free NMD at the dose of 0.1 mg/kg did not reduce the occurrence of peripheral cholinergic signs, stereotypic movements, and tremors. The unencapsulated drug at the dose of 0.1 mg/kg was unable to prevent the installation of the seizure and reduce the mortality rate in mice. As with the mice pretreated with free NMD at the dose of 0.1 mg/kg, free NMD at the dose of 1 mg/kg did not significantly reduce the occurrence of peripheral cholinergic signs, stereotypic movements, and tremors. Moreover, free NMD at the dose of 1 mg/kg was unable to prevent the installation of the seizure and reduce the mortality rate in the mice. Free NMD at doses of 10 mg/kg did not significantly reduce the occurrence of peripheral cholinergic signs or tremors and was not able to prevent the installation of seizures. Free NMD at the dose of 10 mg/kg was able to reduce by 25% of stereotypic movements but was unable to prevent the installation of the seizure and reduce the mortality rate in the mice.

NMD-Lipo at doses of 0.1, 1, and 10 mg/kg did not reduce the occurrence of peripheral cholinergic signs, but decreased stereotypic movements and tremors in the mice. NMD encapsulated into liposomes at all doses tested was able to prevent the occurrence of 100% of the seizures. None of the mice pretreated with NMD-Lipo and subsequently given with pilocarpine died.

4. Discussion

Animal models of seizure have been widely used in research to provide a better understanding of the pathophysiology of the disease, since they reproduce several components of human epilepsies. Pilocarpine-induced seizures is a model commonly used to investigate the anticonvulsant effect of antiepileptic drugs [13].

The administration of high doses of pilocarpine induces seizure activity, followed by a latent seizure-free period preceding the development of spontaneous recurrent focal seizures. The induction of status epilepticus by pilocarpine in rodents leads to neuropathological changes, such as hippocampal sclerosis and mossy fiber sprouting, resembling human temporal lobe epilepsy [14]. In the present study, we investigated the effects of a liposomal formulation containing nimodipine, a Ca^{2+} channel blocker, on susceptibility to seizures induced by pilocarpine in adult mice.

Antiepileptic drugs have different targets such as receptors, synaptic machinery, and ion channels [15]. Previous studies have demonstrated that increased levels of intracellular Ca^{2+} in hippocampal neurons play an important role in the underlying mechanisms of neuronal hyperexcitability that leads to pilocarpine-induced seizures [16]. Research conducted with Ca^{2+} channel blocker NMD at the doses of 1 to 300 mg/kg have suggested that the drug presents anticonvulsant activity on seizures induced by picrotoxin [17], kainic acid [18], aminophylline [19], pentylenetetrazole [20], phenytoin [21], pilocarpine, and lithium-pilocarpine [22–24] in mice and rats. However, in all aforementioned studies, the drug was unable to prevent 100% of seizures.

One of the possible reasons explaining the lack of ability of nimodipine to prevent the installation of seizures in some rodents is that the drug has low bioavailability (4–13%) due to its high first-pass effect in the liver [25]. This hypothesis is strengthened by our data, since NMD-Lipo showed anticonvulsant activity significantly superior to free NMD, suggesting that the encapsulation of the nimodipine into liposomes increases its bioavailability, as well as the anticonvulsant activity of the drug on the animals.

Nimodipine has a high lipophilicity and it can be easily incorporated into the lipid bilayer of the liposomes [26]. NMD-Lipo presented drug encapsulation efficiency of $99 \pm 0.22\%$, showing that the formulation does not present a significant amount of unloaded NMD. The treatment with NMD-Lipo at the doses of 0.1–10 mg/kg was demonstratedto be safe for mice, since these treatments do not cause changes in the hematological and biochemical parameters of the animals are found. Furthermore, NMD-Lipo did not cause any change in the weight of the animals, a significant factor as the reduction in body weight is a simple and sensitive index of toxicity after exposure to a toxic substance [27].

The administration of the liposomal formulation at the doses of 0.1, 1, and 10 mg/kg was able to reduce stereotypic movements and tremors. Moreover, NMD-Lipo prevented the installation of 100% of the pilocarpine-induced seizures and prevented the death of 100% of the mice treated with pilocarpine, showing even better results than the rodents treated with diazepam. The results of the anticonvulsant activity suggest that NMD has a dose-dependent effect. As expected, empty liposomes showed no anticonvulsant activity, suggesting that the liposomes potentiate the anticonvulsant effect of nimodipine.

Animal studies indicate that seizures at an early stage of development can drastically affect the construction of networks of the hippocampus, which can cause the onset of other disorders such as schizophrenia [28]. The decrease in the occurrence of stereotypic movements and tremors and the ability to prevented seizures, and death in rodents constitutes a major advance in drug development against epilepsy. Thus, a formulation that prevents the emergence of seizures appears promising in the epilepsy therapy.

References

[1] O.O. Adeyemi, A.J. Akindele, O.K. Yemitan, F.R. Aigibe, F.I. Fagbo, Anticonvulsant, anxiolytic and sedative activities of the aqueous root extract of *Securidaca longepedunculata* Fresen, J. Ethnopharmacol. 130 (2010) 191–195.
[2] S. Fortini, L. Corredera, A.L. Pastrana, G. Reyes, L. Fasulo, R.H. Caraballo, Encephalopathy with hemi-status epilepticus during sleep or hemi-continuous spikes and waves during slow sleep syndrome: a study of 21 patients, Seizure 22 (2013) 565–571.
[3] P. Bhutada, Y. Mundhada, K. Bansod, P. Dixit, S. Umathe, D. Mundhada, Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice, Epilepsy Behav. 18 (2010) 207–210.
[4] F. Hadizadeh, B. Rahimi, E. Taghiabadi, M. Razavi, G. Karimi, Evaluation of anticonvulsant effect of two novels 4-[1-(4-fluorobenzyl)-5-imidazolyl] dihydropyridine derivatives in mice, Res. Pharm. Sci. 8 (2013) 91–95.
[5] M. Ghasemi, H. Shafaroodi, S. Nazarbeiki, H. Meskar, P. Heydarpour, A. Ghasemi, S.S. Talab, P. Ziai, A. Bahremand, A.R. Dehpour, Voltage-dependent calcium channel and NMDA receptor antagonists augment anticonvulsant effects of lithium chloride on pentylenetetrazole-induced clonic seizures in mice, Epilepsy Behav. 18 (2010) 171–178.
[6] M.J. Bailey, B.A. Hutsell, M.C. Newlan, Dietary nimodipine delays the onset of methylmercury neurotoxicity in mice, Neurotoxicology 37 (2013) 108–117.
[7] N. Bege, T. Renette, T. Endres, M. Beck-Broichsitter, D. Hänggi, T. Kissel, In situ forming nimodipine depot system based on microparticles for the treatment of posthemorrhagic cerebral vasospasm, Eur. J. Pharm. Biopharm. 84 (2013) 99–105.
[8] C. Sun, J. Wang, J. Liu, L. Qiu, W. Zhang, L. Zhang, Liquid proliposomes of nimodipine drug delivery System: preparation, characterization, and pharmacokinetics, AAPS Pharm. Sci. Tech. 14 (2013) 332–338.
[9] P.G. Cadena, M.A. Pereira, R.B.S. Cordeiro, I.M.S. Cavalcanti, B. Barros-Neto, M.C.C.B. Pimentel, J.L. Lima-Filho, V.L. Silva, N.S. Santos-Magalhães, Nanoencapsulation of quercetin and resveratrol into elastic liposomes, Biochim. Biophys. Acta 1828 (2013) 309–316.
[10] L.C.G.A.I. Moreno, G.Z.S. Oliveira, I.M.F. Cavalcanti, N.S. Santos-Magalhães, H.M.L. Rolim, R.M. Freitas, Development and evaluation of liposomal

formulation containing nimodipine on anxiolytic activity in mice, Pharmacol. Biochem. Behav. 116 (2014) 64–68.
[11] B.H. Waynforth, Injection techniques, in: Experimental and Surgical Techniques in the Rat, Academic Press, London, 1980.
[12] L.F.L. Santos, L.F.M. Freitas, S.M.L. Xavier, G.B. Saldanha, R.M. Freitas, Neuroprotective actions of vitamin C related to decreased lipid peroxidation and increased catalase activity in adult rats after pilocarpine-induced seizures, Pharmacol. Biochem. Behav. 89 (2008) 1–5.
[13] W. Zgrajka, D. Nieoczym, M. Czuczwar, J. Kiís, W. Brzana, P. Wlaíz, W.A. Turski, Evidences for pharmacokinetic interaction of riluzole and topiramate with pilocarpine in pilocarpine-induced seizures in rats, Epilepsy Res. 88 (2010) 269–274.
[14] P.E. Schauwecker, Strain differences in seizure-induced cell death following pilocarpine-induced status epilepticus, Neurobiol. Dis. 45 (2012) 297–304.
[15] A.J. Hill, N.A. Jones, I. Smith, C.L. Hill, C.M. Williams, G.J. Stephens, B.J. Whalley, Voltage-gated sodium (Na_V) channel blockade by plant cannabinoids does not confer anticonvulsant effects *per se*, Neurosci. Lett. 566 (2014) 269–274.
[16] Y. Martinez, P. N'Gouemo, Blockade of the sodium calcium exchanger exhibits anticonvulsant activity in a pilocarpine model of acute seizures in rats, Brain Res. 1366 (2010) 211–216.
[17] J. Thomas, The effect of nimodipine on picrotoxin-induced seizures, Brain Res. Bull. 24 (1990) 11–15.
[18] R.P. Paczynski, F.B. Meyer, R.E. Anderson, Effects of the dihydropyridine Ca^{2+} channel antagonist nimodipine on kainic acid-induced lirnbic seizures, Epilepsy Res. 6 (1990) 33–38.
[19] A. Chakrabarti, H.S. Kaur, S.K. Garg, Dose-finding study with nimodipine: a selective central nervous system calcium channel blocker on aminophylline induced seizure model in rats, Brain Res. Bull. 45 (1998) 495–499.
[20] P. Zapater, J. Javaloy, J.F. Román, M.T. Vidal, J.F. Horga, Anticonvulsant effects of nimodipine and two novel dihydropyridines (PCA 50,922 and PCA 50,941) against seizures elicited by pentylenetetrazole and electroconvulsive shock in mice, Brain Res. 796 (1998) 311–314.
[21] C. Hocht, A. Lazarowski, N.N. Gonzalez, J. Auzmendi, J.A.W. Opezzo, G.F. Bramuglia, C.A. Taira, E. Girardi, Nimodipine restores the altered hippocampal phenytoin pharmacokinetics in a refractory epileptic model, Neurosci. Lett. 413 (2007) 168–172.
[22] M.M.F. Marinho, V.M.S. Bruin, F.C.F. Souza, L.M.V. Aguiar, R.S.N. Pinho, G.B.S. Viana, Inhibitory action of a calcium channel blocker (nimodipine) on seizures and brain damage induced by pilocarpina and lithium-pilocarpine in rats, Neurosci. Lett. 235 (1997) 13–16.
[23] M.A. Mikati, G.L. Holmes, S. Werner, N. Bakkar, L. Carmant, Z. Liu, C.E. Stafstrom, Effects of nimodipine on the behavioral sequalae of experimental status epilepticus in prepubescent rats, Epilepsy Behav. 5 (2004) 168–174.
[24] V.S. Nascimento, M.S. D'alva, A.A. Oliveira, R.M. Freitas, S.M.M. Vasconcelos, F.C.F. Sousa, M.M.F. Fonteles, Antioxidant effect of nimodipine in young rats after pilocarpine-induced seizures, Pharmacol. Biochem. Behav. 82 (2005) 11–16.
[25] S.S. Chalikwar, V.S. Belgamwar, V.R. Talele, S.J. Surana, M.U. Patil, Formulation and evaluation of nimodipine-loaded solid lipid nanoparticles delivered via lymphatic transport system, Colloids Surf. B 97 (2012) 109–116.
[26] T. Guan, Y. Miao, L. Xu, S. Yang, J. Wang, H. He, X. Tang, C. Cai, H. Xu, Injectable nimodipine-loaded nanoliposomes: preparation, lyophilization and characteristics, Int. J. Pharm. 410 (2011) 180–187.
[27] S. Thanabhorn, K. Jaijoy, S. Thamaree, K. Ingkaninan, A. Panthong, Acute and subacute toxicity study of the ethanol extract from *Lonicera japonica* Thunb, J. Ethnopharmacol. 107 (2006) 370–373.
[28] G.P. Labbate, A.V. Silva, R.C. Barbosa-Silva, Effect of severe neonatal seizures on prepulse inhibition and hippocampal volume of rats tested in early adulthood, Neurosci. Lett. 568 (2014) 62–66.