

## Evaluation of anticonvulsant effect of two novels 4-[1-(4-fluorobenzyl)-5-imidazolyl] dihydropyridine derivatives in mice

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### Abstract

In this study the anticonvulsant effect of two dihydropyridine derivatives [diethyl -1,4- dihydro -2,6- dimethyl -4-(4- fluoro benzyl-2- methylthio -5- imidazolyl)-3,5- pyridine dicarboxilat (**A**) and diethyl -1,4- dihydro -2,6- diethyl -4-(4- fluoro benzyl-2- methylthio -5- imidazolyl)-3,5- pyridine dicarboxilat (**B**)] by pentylenetetrazole (PTZ) and electroshock in mice was evaluated. The latency and HLTE (hind limb tonic extensions), the duration of HLTE and the mortality protection in pentylenetetrazole test and the HLTE duration in electroshock test were assessed. Both compounds had significant differences with negative control in all doses used. There was no significant difference between nifedipine and **B** (96.7 and 169.2 mg/kg doses) in the starting point of HLTE and between nifedipine and **A** (62.2 and 108.9 mg/kg doses) in the duration of HLTE in the PTZ test. Also, there was no significant difference between nifedipine and **B** (96.7 and 169.2 mg/kg doses) and **A** (62.2 and 108.9 mg/kg doses) in electroshock test. All doses of **A** and **B** and nifedipine showed less effect than phenytoin and valproate. This study showed that both **A** and **B** have anticonvulsant activity in the PTZ-induced seizure model and the MES test. These compounds, thus, might be useful in the petit mal and grand mal epilepsy.

**Keywords:** Dihydropyridine; Pentylenetetrazole; Electroshock; Seizure; Calcium channel blocker; Anticonvulsant

### INTRODUCTION

Epilepsy is the most common neurological disease which is identified by recurrent spontaneous seizures and up to 5% of the world population develops epilepsy in their life (1-3). Although different antiseizure medications are available to alleviate epileptic disorders in patients, there are still around 30% of patients medicated incompletely. Therefore, investigation of new antiseizure drugs which allow more efficient control seizure and its associated problems seems imperative (4,5).

Due to the adverse effects, toxicity and teratogenic effects of current antiepileptic drugs in the treatment of epilepsy (1), calcium channel blockers as antiepileptic agents have recently been considered. Anti-epileptic drugs

with less adverse effects and more activities in resistant patients are greatly needed. It has been shown that calcium ion is an important factor for the induction of epilepsy. Therefore, calcium channel blockers such as nimodipine or nifedipine may be considered as complementary anticonvulsant drugs (6). Since dihydropyridines show antihypertensive activity more than hypotensive effect, it is not a considerable limiting factor for use as a complementary antiepileptic drugs (7,8). The anticonvulsant effect of calcium antagonists through the inhibition of  $Ca^{2+}$  entry via voltage-dependent L-channels has been investigated in some in vitro and in vivo studies (9). Samzadeh-Kermani and coworkers showed that lipophilic 2-(4-chlorophenyl) -4-thiazolyl -1, 4- dihydropyridines possess anti-

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convulsant activity (6). In addition, anticonvulsant effect of lipophilic 4-imidazolyl-1, 4-dihydropyridines derivatives has also been investigated. These derivatives indicated an increase in seizure threshold as compared with that of control. Anticonvulsant effect of these derivatives depended on their lipophilicity and the time needed for maximum effect decreased with increasing the lipophilicity (10). Some studies have demonstrated that anticonvulsant property of nimodipine and a novel dihydropyridine, PCA 50922, was similar to that of clonazepam (9,11). In another study, nimodipine, nifedipine, and amlodipine exhibited anticonvulsant effect in a dose dependent manner (12).

Because of neurologic adverse effects of calcium antagonists and interactions of antiepileptic drugs with some of the calcium channel blockers, dihydropyridines are considered as candidates for the treatment of epileptic disorders in clinical trials (10, 13-15).

Two novel dihydropyridine compounds, [diethyl -1,4- dihydro -2,6- dimethyl -4-(4-fluoro benzyl-2- methylthio -5- imidazolyl)-3,5- pyridine dicarboxilat (**A**) and diethyl -1,4- dihydro -2, 6- diethyl -4- (4- fluoro benzyl-2- methylthio -5- imidazolyl) -3, 5- pyridine dicarboxilat (**B**)] were synthesized by Hadizadeh and coworkers and showed the calcium channel antagonist activities and decreased the contraction of the rat isolated ileum preparations in comparison with reference drug nifedipine (16). Because of higher lipophilicity and better penetration to CNS, we studied the effect of these compounds against seizure elicited by pentylenetetrazole (PTZ) and electroshock in mice.

## MATERIALS AND METHODS

### Chemicals

[diethyl -1,4- dihydro -2,6- dimethyl -4-(4-fluoro benzyl-2- methylthio -5- imidazolyl)-3, 5- pyridine dicarboxilat (**A**) and diethyl -1,4- dihydro -2, 6- diethyl -4- (4- fluoro benzyl-2- methylthio -5- imidazolyl)-3, 5- pyridine dicarboxilat (**B**)] were synthesized by Hadizadeh and coworkers (16). Phenytoin was provided by Loghman pharmaceutical and hygienic Co. (Tehran, Iran). Sodium valproate

obtained from Rouz Darou Pharmaceutical Co. (Tehran, Iran). Nifedipine was provided by Zahravi Pharmaceutical Co. (Tabriz, Iran), and Pentylenetetrazole purchased from Aldrich (Germany).

### Animals

The study was performed on male albino mice weighing 25-30 g. Animals were housed in a ventilated room under a 12/12 h light/dark cycle at  $24 \pm 2^\circ\text{C}$  and had free access to water and food. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences Ethical Committee Acts.

### Acute toxicity

Different doses of **A** and **B** were injected intraperitoneally into groups of six mice. The number of deaths was counted at 48 h after the treatment.  $\text{LD}_{50}$  values and corresponding confidence limits were determined by the Litchfield and Wilcoxon method (PHARM/PCS Version 4).

### Anticonvulsant activity

#### PTZ-induced seizure

The mice were divided into ten groups of five animals each. They were given **A** (15.6, 62.2 and 108.9 mg/kg) and **B** (24.2, 96.7 and 169.2 mg/kg), nifedipine (30 mg/kg), sodium valproate (250 mg/kg) as positive control, and DMSO and normal saline as negative controls, intraperitoneally 60 min before administration of 90 mg/kg PTZ. The starting time of the first seizure and (hind limb tonic extensions (HLTE), as well as the percentage of protection against incidence of the mortality were recorded (17,18).

#### Maximal electroshock seizure (MES) test

The mice were divided into ten groups of five animals each. They were given **A** (15.6, 62.2 and 108.9 mg/kg) and **B** (24.2, 96.7 and 169.2 mg/kg), nifedipine (30 mg/kg), phenytoin (30 mg/kg) as positive control, and DMSO and normal saline as negative controls, intraperitoneally 30 min before induction of MES. Then, the stimulus train was applied via ear-clip electrodes (sinusoidal pulses 150 mA and 60 Hz for 0.2 s) by means of a constant current stimulator (Digital Electroshock Model

150, EghbalTeb Co., Mashhad, Iran). A drop of 0.9% saline solution was poured on each ear of animals prior to placing the electrode. HLTE duration was determined (17,18).

### Statistical analysis

The data were expressed as mean values  $\pm$  S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer. Discrepancies with  $P < 0.05$  were considered significant.

## RESULTS

### Acute toxicity

LD50 values of the **A** and **B** were 155.57 mg/kg body wt. (95% CL: 137.69- 175.76) and 241.78 mg/kg body wt. (95% CL: 226.83- 257.72), respectively.

### PTZ-induced seizure

In the PTZ-induced seizure, both **A** and **B** had significant differences with negative controls in all tested doses. Administration of all doses of **A** and **B** 60 min before the injection of PTZ, increased the starting time of the first seizure compared to the negative control group ( $P < 0.001$ ) (Table 1). The results of the starting time of HLTE showed that there was no significant difference between nifedipine and **B** (96.7 and 169.2 mg/kg doses) (Table 1 and 2.). Also, there was no significant difference between nifedipine and both **A** (62.2 and 108.9 mg/kg doses) and **B** (96.7 and 169.2 mg/kg doses) in the duration of HLTE ( $P > 0.05$ ) (Table 1). The effect of all doses of **A** and **B** and nifedipine was less than sodium valproate (Table 1 and 2). The protective effect against lethality was 40% at the highest

**Table 1.** Effects of dihydropyridine **A** and **B** on PTZ-induced seizure in mice.

Treatment	Latency (s)	HLTE starting time (s)	HLTE duration (s)	Protection against mortality (%)
Normal Saline (10 ml/kg)	36.8 $\pm$ 3.73	98.8 $\pm$ 11.64	23.4 $\pm$ 1.43	0
DMSO (10 ml/kg)	36 $\pm$ 3.84	85.4 $\pm$ 9.04	24.8 $\pm$ 0.58	0
Sodium valproate (250 mg/kg)	3600 $\pm$ 0.00***	3600 $\pm$ 0.00***	0.00 $\pm$ 0.00 ***	100
Nifedipine (30 mg/kg)	155.6 $\pm$ 10.81***	1079.8 $\pm$ 44.4***	9.8 $\pm$ 0.58 ***	20
<b>A</b> (15.6 mg/kg)	84.8 $\pm$ 4.05***	406.8 $\pm$ 71.2 ***	16.6 $\pm$ 0.98 ***	0
<b>A</b> (62.2 mg/kg)	107.2 $\pm$ 5.40***	836.75 $\pm$ 30.77 ***	14.3 $\pm$ 1.76 ***	40
<b>A</b> (108.9 mg/kg)	116.4 $\pm$ 5.48***	609.4 $\pm$ 22.42***	11.6 $\pm$ 0.98 ***	40
<b>B</b> (24.2 mg/kg)	91.6 $\pm$ 4.54***	703 $\pm$ 44.5***	17.6 $\pm$ 0.92 ***	0
<b>B</b> (96.7 mg/kg)	111.8 $\pm$ 5.64***	1231.6 $\pm$ 76.96***	12.00 $\pm$ 0.83 ***	60
<b>B</b> (169.2 mg/kg)	123 $\pm$ 10.07***	1150 $\pm$ 34.95***	14.25 $\pm$ 0.85***	40

All agents were administered 60 min before the injection of PTZ, Negative Controls: Normal saline and DMSO. Positive controls: Nifedipine and Sodium valproate. Values are mean  $\pm$  SEM for 5 mice; \*\*\*p < 0.001, as compared with negative controls, Tukey-Kramer.

**Table 2.** Effects of dihydropyridine **A** and **B** on MES-induced seizure in mice.

Treatment	HLTE duration (s)
Normal Saline (10 ml/kg)	25.0 $\pm$ 0.7
DMSO (10 ml/kg)	26.2 $\pm$ 1.4
Phenytoin (30 mg/kg)	0.00 $\pm$ 0.00 ***
Nifedipine (30 mg/kg)	11.6 $\pm$ 0.9 ***
<b>A</b> (15.6 mg/kg)	18.8 $\pm$ 1.2 ***
<b>A</b> (62.2 mg/kg)	12.6 $\pm$ 0.8 ***
<b>A</b> (108.9 mg/kg)	11.6 $\pm$ 1.1 ***
<b>B</b> (24.2 mg/kg)	18.6 $\pm$ 1.4 ***
<b>B</b> (96.7 mg/kg)	11.4 $\pm$ 0.7 ***
<b>B</b> (169.2 mg/kg)	11.0 $\pm$ 0.7 ***

All agents were administered 30 min before induction of MES, Negative Controls: Normal saline and DMSO. Positive controls: Nifedipine and Phenytoin. Values are mean  $\pm$  SEM for 5 mice; \*\*\*p < 0.001, as compared with control, Tukey-Kramer.

dose of **A** and 60% at the dose of 96.7 mg/kg of **B** (Table 1).

#### **Maximal electroshock seizure test**

In the MES test, both **A** and **B** showed significant differences with negative control in all doses. Also, there was no significant difference between nifedipine and **B** (96.7 and 169.2 mg/kg doses) and **A** (62.2 and 108.9 mg/kg doses) in the duration of HLTE ( $P>0.05$ ). The effect of all doses of **A** and **B** and nifedipine was less than that of phenytoin (Table 2).

### **DISCUSSION**

This study indicated that both **A** and **B** have moderate anticonvulsant activity. Our results exhibit that these compounds had anticonvulsant effect on the PTZ-induced seizure model. In general, compounds with the anticonvulsant activity in the petit mal epilepsy, are effective in PTZ-induced seizure model (19). Thus, both **A** and **B** may be useful in petit mal epilepsy. On the other hand, since dihydropyridine compounds exhibit moderate anticonvulsant property in MES model, they can also exert a protective activity against the grandmal epilepsy. Therefore, compounds which have anticonvulsant activity in MES may be considered as an effective compounds against the grand mal epilepsy (19).

We have shown that the anticonvulsant effect of **B** is more than **A** which is due to its greater lipophilicity imparted through the lengthening of its chain. The results of the present study are in agreement with previous study reported that increasing the lipophilicity of the molecule as in the case of 4-imidazolyl-1, 4-dihydropyridines, decreases the time needed for maximum anticonvulsant activity (10).

With regard to LD50 values, **A** is more toxic than **B**. It seems that the toxicity of these compounds is related to SAR, pharmacokinetic and effects on different organs such as central nervous system, cardiovascular, liver and kidney (20-21). It was shown that calcium antagonists possess anticonvulsant effect beside cardiovascular activities (6, 23-24). Since dihydropyridine

receptors exist in the brain, central activity of dihydropyridines like nimodipine and nifedipine are demonstrated through their anticonvulsant effect induced by ischemia, bicuculline, electrical cortical shock, pentylentetrazole, nitrous oxide as well as alcohol abstinence syndrome or high-pressure exposure (22,25).

In this study the anticonvulsant properties of two novel dihydropyridine derivatives was observed and our results are in consistence with other studies (6, 9-12).

The antiepileptic effect of dihydropyridines and other calcium channel blockers may be related to the inhibition of calcium L type channels as well as T type channels. Neuronal calcium channel inhibition may be important in protecting seizure disorders (22,26). Furthermore, the other mechanisms involved in anticonvulsant effect of dihydropyridine calcium antagonists include: inhibition of N-methyl-D,L-aspartate (NMDLA) receptors (25) and some important interactions between calcium antagonists like dihydropyridines and adenosine (22).

### **CONCLUSION**

In conclusion, the results presented here show that dihydropyridine calcium channel blockers may influence some neuronal functions such as anticonvulsant activity. This could lead to the use of some novel dihydropyridines as complementary agents for treating epilepsy to reduce the adverse effects and to increase their efficiency.

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