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Effect of Diltiazem on Dichlorvos-Induced Seizure in Mice

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Abstract: Dichlorvos a synthetic organophosphate poisons are the property of insecticide. These toxins as insecticides in agriculture and medicine for animals and the destruction of ectoparasites can be used. Studies have shown that Dichlorvos creation seizure effects in different animals. Diltiazem, calcium channel blockers, widely used for treatment of cardiovascular diseases. Studies have shown that the calcium channel blockers are anti-convulsant effects in different animal models. The aim of this study was to determine the effect of Diltiazem on Dichlorvos-induced seizures in mice. In this experiment, the animals were received different doses of Diltiazem (2.5, 5, 10, 20 and 40 mg kg^{-1}) intraperitoneally 30 min before intraperitoneal injection of Dichlorvos (50 mg kg^{-1}). After Dichlorvos injection, clonic and tonic seizures and death was investigated. Results showed that Diltiazem dose dependently reduced the severity of Dichlorvos-induced seizures so that Diltiazem dose 10 and 40 mg kg^{-1}, respectively the lowest ($p<0.05$) and highest ($p<0.01$) had anti-convulsant effects. The anti-convulsant activity of Diltiazem suggests that possibly due to antagonistic effect on voltage-dependent calcium channel.

Key words: Dichlorvos, Diltiazem, seizures, mice, injection, animals, toxins

INTRODUCTION

Epilepsy is one of the major neurological diseases in humans and about 1% of the population is involved. It has been shown that epileptic seizure occurs due to occasional discharges in nerve tissue. It is recognized that occasional changes in reversible neuronal function causing brain electrical activity. In some cases, the seizure occurs due to the entry of calcium ions into nerve cells and reducing intracellular calcium concentration in some epileptic animal models has inhibitory effect on seizures. During seizures increased intracellular calcium ion concentration and but extra cellular calcium concentration decreases (Khanna *et al*., 2000; Van Luijtelaar *et al*., 1995). Calcium channel antagonists for the treatment of hypertension were produced in the year 1980. Use of these drugs over time to treat other diseases was developed such as treatment of angina, supraventricular tachycardia attack, hypertrophic cardiomyopathy, pulmonary hypertension and migraine. Recently have shown that calcium channel blockers may have anti-convulsant effects in some animal models. Calcium channel blockers inhibit calcium ion flow through L-type calcium channels sensitive to voltage (Kulak *et al*., 2004). It has been shown that calcium channel inhibitors in models of nerve tissue in a large protective effect (Mikati *et al*., 2004). Have also reported that calcium channel inhibitors on the anti-convulsant effects of some models have (Chakrabarti *et al*., 1998; Marinho *et al*., 1997) but in all animal models of seizures has not demonstrated these effects (Gasior *et al*., 1996; Khosla and Pandhi, 2000). Also, in rats anti-convulsant effects of calcium channel inhibitors shown but seizure agent has not Dichlorvos. Some medications such as anti-convulsants phenytoin and carbamazepine effect by inhibiting sodium channels directly and indirectly by preventing the flow of calcium from the membranes of neurons and reduction of excessive concentration of intracellular calcium. Specific drugs used to treat epilepsy are absence seizure kind of like channels as T-type calcium in thalamic neurons are blocked. This reduced calcium ion concentration of the important goals in development is neuroprotective and anti-epileptic drugs (Kulak *et al*., 2004; Van Luijtelaar *et al*., 1995). Calcium entry into neurons play an important role in creating the seizures and calcium channel inhibitors have different effects on health including cardiovascular diseases, migraine and headaches caused by vascular changes, nerve regeneration and neuronal regenerative processes (Khanna *et al*., 2000). So, it seems calcium channel inhibitors used to treat seizures can be useful. Results of these studies for the anti-convulsant effects of calcium channel inhibitors suggests therefore, likely to Diltiazem reduce Dichlorvos-induced seizures. Since, no research based on the combined effect of these seizures from Dichlorvos there in this case study seems necessary. Insecticide use in agriculture and veterinary medicine as strange since World War II and grew during the past 20 years has reached its highest rate constant. While the main consumers of agricultural insecticide industry is also

large quantities of other industries use them and their applications in and around homes is considerable. Most of insecticide residues on the remaining products and people exposed to low doses of chemicals through the foods. Numerous incidents of acute insecticide poisoning caused by eating food that mainly followed during storage or transportation had been infected was created (Goodman *et al*., 2001). Including the insecticide which are potential toxicity are organophosphate. One of the organophosphate is Dichlorvos. The aim of this study was to determine the effect of Diltiazem (calcium channel antagonist) on Dichlorvos-induced seizures.

MATERIALS AND METHODS

Male mice NMRI, weighing between 25-30 g and maintenance of Laboratory Animals Breeding Center of Tabriz Islamic Azad University purchased and were kept in room temperature, light and humidity constant. Animals access to food and water were freely. All tests were performed between 10-16 h. Dichlorvos and Diltiazem both were solved in Twin 80 (5%). Animals were divided randomly and placed in treatment groups (each group n = 10). Diltiazem and Twin 80 were administered intraperitoneally with constant volume and by weight per animal. To remove the effect of injection volume on seizures, all drugs and Twin 80 at 10 mL kg^{-1} was set. First, seizures was assessed in animals receiving Dichlorvos and then evaluated the effect of Twin 80 on Dichlorvos-induced seizures with the above injection, 30 min before the seizure was determined. More experimental animals, different doses of Diltiazem (2.5, 5, 10, 20 and 40 mg kg^{-1}) received 30 min before the intraperitoneal injection of Dichlorvos. To create seizures, mice received Dichlorvos (50 mg kg^{-1}) intraperitoneally and then the animals treated for 120 min were recorded by video camera. Films from the following four behaviors were recorded; starting time of clonic seizures after injection of Dichlorvos (sec), generation time of death after Dichlorvos injection (sec), mortality after injection of Dichlorvos (persentage) and type of seizures induced by injection of Dichlorvos (percentage). After testing data as the mean±SEM expression and to analyze data, ANOVA followed by Tukey multiple comparison tests were used. Value of $p<0.05$ to determine significance between groups was considered.

RESULTS AND DISCUSSION

Effect of Twin 80 as solvent on Dichlorvos-induced seizures showed that this substance has no significant effect on seizures. Therefore, the results had not presented in graphs and tables have been avoided. Effect of different doses of Diltiazem (2.5, 5, 10, 20 and 40 mg kg^{-1}) on Dichlorvos-induced seizures showed that this drug dose-dependently reduced the Dichlorvos-induced seizures (Fig. 1 and 2). Most anti-convulsant effect of Diltiazem on the mortality and severity of seizures with a dose of 40 mg kg^{-1} was observed (Fig. 3 and Table 1). In this study, Dicholorvos cause clonic and tonic seizures and ultimately death. After the mice received intraperitoneal dichlorvos, some degree of tremor and excessive activity showed that over time the symptoms became more severe and cause death. In this study, Diltiazem dose dependently reduced clonic and tonic seizures and deaths from Dicholorvos. The results

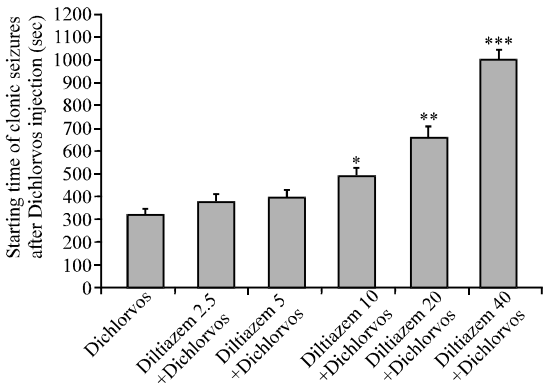

Fig. 1: Effect of different doses of Diltiazem on the starting time of clonic seizures after injection of Dichlorvos (sec). mean±SEM any form of graphs are presented. *p<0.05, **p<0.01 and ***p<0.001 compared with Dichlorvos group

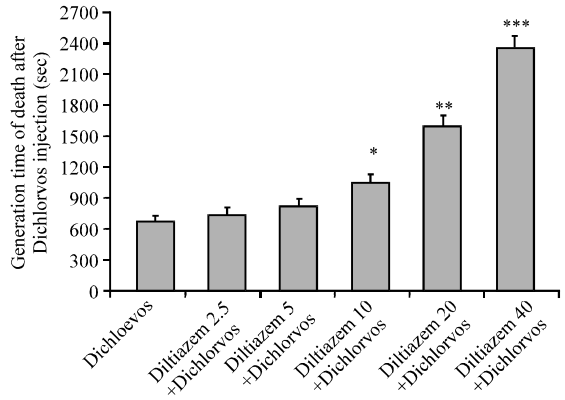

Fig. 2: Effect of different doses of Diltiazem on generation time of death after Dichlorvos injection (sec). Mean±SEM any form of graphs are presented. *p<0.05, **p<0.01 and ***p<0.001 compared with Dichlorvos group

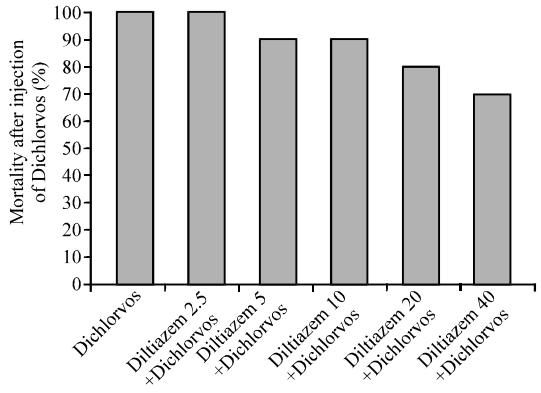

Fig. 3: Effect of different doses of Diltiazem on the mortality after injection of Dichlorvos (%)

Table 1: Effect of different doses of Diltiazem on the type of seizures induced by Dichlorvos injection (%)

Type of seizures (%) group	Low	Moderate	Severe
Dichlorvos	0	0	100
Diltiazem 2.5+Dichlorvos	0	10	90
Diltiazem 5+Dichlorvos	0	20	80
Diltiazem 10+Dichlorvos	10	40	50
Diltiazem 20+Dichlorvos	30	30	40
Diltiazem 40+Dichlorvos	40	50	10

showed that the results of different researchers before treatment with calcium channel antagonists, seizure activity creation of different materials down to is in agreement. If nimodipine in preventing tonic convulsions caused by PTZ (Khanna *et al*., 2000), Aminophyline (Chakrabarti *et al*., 1998) and pilocarpine (Marinho *et al*., 1997) have a protective effect but unlike the above models in all tests anti-convulsant effect has been observed. For example, Kainic acid induced seizures, administration of nimodipine before this material could not reduce seizures (Mikati *et al*., 2004). Another study showed that calcium channel blockers with values >80 mg kg^{-1} could inhibit tonic seizures from chemicals including PTZ in mice and rats (Gasior *et al*., 1996; Wurpel and Iyer, 1994) but later showed that high doses of calcium channels blockers cause systemic and cardiac disorders such as a sharp reduction in coronary blood pressure, decreased movements, imbalance and headache relief (Kulak *et al*., 2004; Van Luijtelaar *et al*., 1995). Epilepsy in patients who were resistant to treatment have reported that nimodipine in an uncontrolled study, seizure frequency is reduced (De Falco *et al*., 1992) but in another study that two strains were unaware controls, no anti-convulsant effects was observed by nimodipine (Larkin *et al*., 1991). Other problems prescription drug, long-term administration of drugs with low prescribed intervals (3-4 times a day to several weeks) and side effects include headache and hypotension, pronounced the man was from animal models. However after 24 and 72 h of administration of nimodipine, percent of alpha and theta waves was increased and vice versa percent in delta waves electroencephalogram was reduced (Kulak *et al*., 2004; Larkin *et al*., 1991). Other studies have shown that the anti-convulsant effects of calcium channel blockers with other anti-epileptic drugs, increases. For example, in mice and rats with concurrent administration of nimodipine with other drugs can be decreased PTZ-induced tonic seizures, seizures resulting from sound and relieve the electroshock (Gasior *et al*., 1996; Khosla and Pandhi, 2000; Mikati *et al*., 2004). Calcium channels blockers in experimental seizures by ischemia, bicuculine, electrical cortical shocks, nitrous oxide and alcohol withdrawal syndrome is caused due have anti-convulsant effects (Kriz *et al*., 2003). In another study, calcium channel blockers such as verapamil, nifedipine and Flunarizine to prevent of penicillin-induced seizures and electroencephalogram range have changed (Kriz *et al*., 2003). Calcium channel inhibitors on seizures induced by N-Methyl-D, L-Aspartate (NMDLA) and dihydropyridine calcium channel agonist BAY K 8644 have been effective (Palmer *et al*., 1993; Van Luijtelaar *et al*., 1995). Another study on rats have shown that nimodipine in animal models of seizures, nerve discharge from BAY K 8644 and reduced the decrease in spike-wave EEG (Van Luijtelaar *et al*., 1995). Also shown that this drug is ischemic brain damage has protective effects (Kriz *et al*., 2003). These studies suggest that protective effects of calcium channel antagonists probably due to blocking L-type calcium channels during seizures. These drugs inhibit voltage-dependent calcium channels in seizures, the increase in intracellular calcium to prevent. Well marked that increased Ca^{2+} into the cell in the incidence of certain types of seizures plays a role (Khanna *et al*., 2000) also marked the loss of calcium outside the cell with reduced flow of calcium from the membranes of neurons for several seconds the discharge of neurons that causes seizures be prevented and the threshold increases (Mc Namara, 1992). Some of the other anti-epileptic drugs such as phenytoin and carbamazepine with a direct effect on neuronal sodium channels act directly or indirectly the flow of calcium ions from the membranes of neurons are inhibited (Kulak *et al*., 2004; Van Luijtelaar *et al*., 1995). So, it is likely that calcium channel antagonists to act with similar mechanisms. Also have shown that calcium channel blockers may inhibit calcium, sodium, chloride, potassium and calcium-dependent glutamate channels (Van Luijtelaar *et al*., 1995).

CONCLUSION

This study showed that Diltiazem (voltage-dependent calcium channel antagonist type L) decreased

clonic and tonic seizures from Dichlorvos in mice is probably the main mechanism anti-convulsant related to block calcium channels and reduce calcium flow with in neurons. Of course, that this could be generalized to humans rather than question and anti-convulsant effects of calcium channel antagonists in humans, further investigation is needed.

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