Cromakalim, a Potassium Channel Opener, Ameliorates the Organophosphate and Carbamate-Induced Seizure in Mice

Sattar Ostadhadi[1,2], Abouzar Moradi[3], Samira Zolfaghari[2], Vahid Nikoui[4], and Ahmad Reza Dehpour[1,2]

[1] *Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[2] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[3] *Department of Periodontics, School of Dentistry, Alborz University of Medical Sciences, Alborz, Iran*
[4] *Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran*

Received: 02 Nov. 2016; Accepted: 12 Dec. 2017

Abstract- Organophosphates (OPs) and carbamates are acetylcholine esterase inhibitors (AChEIs), which can cause seizure and lethality. Anticonvulsant properties of potassium channel openers including cromakalim have been determined in previous studies. In the present experiment, the possible effect of cromakalim on the convulsion and death induced by OPs and carbamates was studied in mice. Dichlorvos (an OP, 50 mg/kg) and physostigmine (a carbamate, 2 mg/kg) were used to induce seizure in animals. Cromakalim at doses of 0.1, 10, and 30 µg/kg was injected 30 min before dichlorvos and physostigmine, and 5 min before glibenclamide (a potassium channel blocker, 1 mg/kg) administration. All injections were performed intraperitoneally. After drugs administration, the onset of convulsion, death, the severity of seizure, and rate of mortality were investigated. Results revealed that both dichlorvos and physostigmine induced seizure activity and lethality in 100% of the animals. Cromakalim at doses of 0.1, 10, and 30 µg/kg significantly increased the latency of both seizure and death (P<0.05). Also, cromakalim decreased the mortality rate induced by dichlorvos and physostigmine (P<0.05). On the other hand, glibenclamide blocked all aspects of the anticonvulsant effect of cromakalim (P<0.05). This study revealed for the first time that cromakalim (a K_{ATP} channel opener) diminishes the seizure and death induced by dichlorvos and physostigmine in mice, and introduces a new aspect to manage the patients who suffer from OPs/carbamates-induced seizure.

Acta Med Iran 2018;56(1):14-20.

Keywords: Organophosphates; Carbamates; Potassium channel opener; Seizure; Mice

Introduction

Organophosphates (OPs) and carbamates have primarily been employed as pesticides in agriculture (1). Some kinds of OPs have shown therapeutic properties and have been used as drugs for the treatment of some neurologic disorders including Alzheimer's disease (2). Both agricultural and medical usages of OPs and carbamates are attributed to their ability to disrupt the function of the cholinergic nervous system by inhibiting acetylcholine esterase (AChE) (3). This enzyme is responsible for the hydrolysis of acetylcholine (ACh) in physiological condition; thus, its blockade leads to the ACh accumulation in the nervous system (2).

In humans, poisoning with OPs/carbamates may result in convulsions (4). The cellular mechanisms by which these agents induce seizure are not fully understood (5). Because of that, there is no effective treatment to manage patients suffering from OPs/carbamates-induced seizure. Although past studies, relying on the disruption of the cholinergic system due to these chemicals, suggest the use of atropine, oximes, and benzodiazepines to control this kind of seizure, all such treatments are not satisfactory and showed severe adverse effects (6,7). Hence, the mechanisms of action of seizure induction by OPs and carbamates must be explained and the importance of such studies to discover new therapies and drugs with favorable pharmacologic properties and side effect profile is unquestionable.

Potassium ion (K^+) channels may play an important role in the control of all features of neuronal excitability including resting membrane potential (8). The opening

Corresponding Author: A.R. Dehpour
Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 88973652, Fax: +98 21 88973652, E-mail address: dehpour@yahoo.com

of K^+-channels is expected to hyperpolarize neurons and inhibit action potential firing and prevent hyper-synchronous neuronal discharges, which occur during a seizure (9,10). Therefore, K^+-channel opening is one of the potential anti-epileptic mechanisms (11,12). On the other hand, past reports have shown that K_{ATP} channels, one of the different types of K^+ channel family, play an important role in the management of seizure threshold in several *in vitro* and *in vivo* models (13,14). These findings have been confirmed by other clinical trials (15). However, there is no study to show the protective effects of K^+-channel openers on seizure induced by OPs and carbamates.

This study was carried out to investigate the possible effect of cromakalim (a potassium channel opener) on OP and carbamate-induced seizure in mice. Therefore, dichlorvos and physostigmine sulfate were used in this experiment as an OP and carbamate, respectively, to induce seizure in mice, and the possible effect of potassium channel openers on convulsion was examined using cromakalim.

Materials and Methods

Animals

In total, 120 male Naval Medical Research Institute (NMRI) mice weighing 20-26 g were used in the experiments. Animals were housed in a room with controlled temperature (21-22° C), light (12-h light/dark cycle), and humidity (50±10%). Animals were allowed free access to standard laboratory, food and water. Assignment of subjects to experimental groups (n=10 in each group) was randomized. The tests were performed between 9 a.m. and 1 p.m. The experimental procedures used throughout this study were in compliance with the guidelines for the Care and Use of Animals and were approved by the Local Ethics Committee on Animal Experimentation of Tehran University of Medical Sciences.

Drugs

The following drugs were used in the experiments: dichlorvos (50 mg/kg) as an OP, physostigmine sulfate (2 mg/kg) as a carbamate, cromakalim (0.1, 10, and 30 µg/kg) as a potassium channel opener, and glibenclamide (1 mg/kg) as a potassium channel blocker. As previously reported, this dose of glibenclamide (1 mg/kg) and its vehicle (dimethyl sulfoxide [DMSO]), did not alter the plasma glucose levels, significantly (9). Dichlorvos (2, 2-chlorovinyl dimethyl phosphate) was prepared from the Institute of Organic Chemistry (Waster green, Iran). Physostigmine sulfate, cromakalim, and glibenclamide were purchased from Sigma-Aldrich (USA). Dichlorvos was mixed with powdered acacia and distilled water in a dry mortar, as described previously (16). Glibenclamide was dissolved in DMSO. Physostigmine and cromakalim were dissolved in saline. Dosages of all drugs were based on the weight of their salt forms.

Experimental design

Dichlorvos and physostigmine were administrated intraperitoneally (*i.p.*) to animals to induce seizure. Two experimental groups (dichlorvos-treated and physostigmine-treated) were divided into three categories:

To investigate the possible role of potassium channels in the seizure induced by OP and carbamate, we used cromakalim and glibenclamide:

(a) Cromakalim (0.1, 10, and 30 µg/kg) was administrated intraperitoneally 30 minutes before OP/carbamate administration.

(b) Glibenclamide (1 mg/kg) was administrated intraperitoneally 30 minutes before OP/carbamate injection.

(c) Further, to determine the effect of cromakalim in seizure induced by OP/carbamate, cromakalim at dose 30 µg/kg (as an effective dose) was administrated 5 minutes before glibenclamide (10).

Behavioral assessments

Animals were monitored for 120 minutes after drug injections. Seizures were evaluated and scored based on the staging system defined by McLean *et al.*, (5,17). It will define stage 0 if there is no abnormal behavior; In stage 1 some abnormal behaviors including salivation, chewing, and pawing of mouth were observed; Stage 2, dazed appearance, intermittent motionlessness, tremor, and/or bobbing of the head was observed; Stage 3, like Stage 2, random and/or generalized jerks are seen; Stage 4, intermittent rearing on hind legs with forepaws extended without falling; Stage 5 is same as the stage 4, with falling to the side or rear; Stage 6, status epilepticus was observed. Stages 1-3 and 4-6 were considered as sub-convulsive and convulsive behaviors, respectively. The latency of seizures after injection of drugs (in seconds), the latency to onset of death within one hour (in seconds), mortality after injection (percentage) within one hour, and stages of seizures induced by injection of drugs (percentage) were recorded.

Data analysis

Data were analyzed using GraphPad Prism software, version 5 (USA). Comparisons between experimental and control groups were performed by one-way ANOVA followed by Tukey's *post-hoc* when appropriate. A value of $P<0.05$ was considered to be significant.

Results

Dichlorvos and physostigmine administration cause convulsion and death in mice

Both dichlorvos (50 mg/kg, *i.p.*) and physostigmine (2 mg/kg, *i.p.*) result in seizure activity in mice (Table 1). In these groups, convulsion and death (Table 1) occurred in 100% of the animals.

Effect of cromakalim on the onset of seizure/death induced by dichlorvos and physostigmine

Pretreatment with cromakalim at doses of 10 µg/kg ($P<0.05$ for physostigmine, $P<0.01$ for dichlorvos) and 30 µg/kg ($P<0.01$ for dichlorvos and physostigmine) increased the latency time of clonic seizure (Figure 1) and also prevented the dichlorvos- or physostigmine-induced seizures in mice (Table 1).

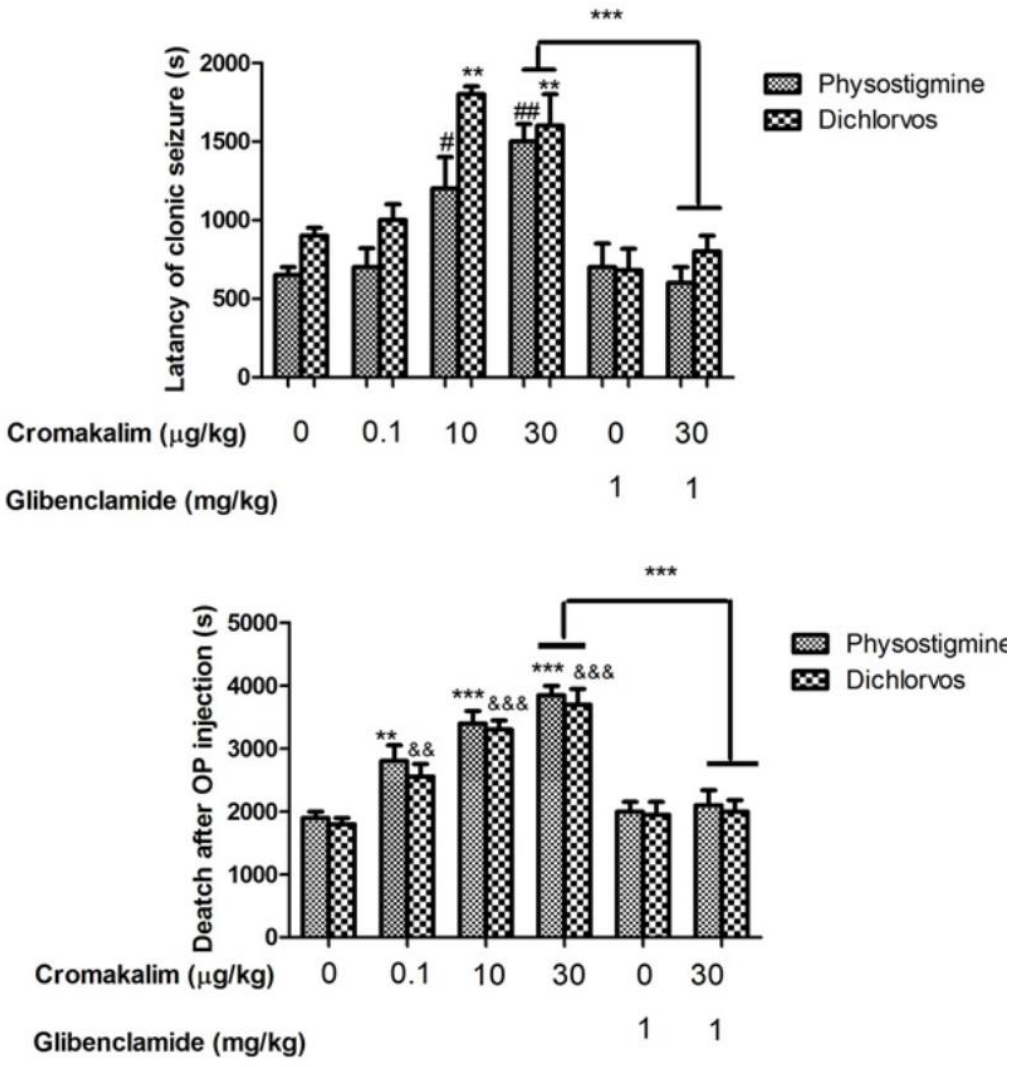

Figure 1. Effect of different doses of cromakalim (0.1, 10, and 30 µg/kg) and glibenclamide (1 mg/kg) on the starting time of seizure after dichlorvos (50 mg/kg) and physostigmine (2 mg/kg) injection

Effect of cromakalim (30 µg/kg) and glibenclamide (1 mg/kg) co-administration on the onset of seizure in mice). Values are shown as mean+S.E.M. of 10 animals. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared physostigmine-injected group (control). $^{**}P<0.01$ compared dichlorvos-injected group (control). $^{***}P<0.001$ compared to cromakalim (30 µg/kg) + glibenclamide (1 mg/kg) -administrated group.

Table 1. Effect of different doses of cromakalim and glibenclamide on the stages of seizure induced by dichlorvos (50 mg/kg) and physostigmine (2 mg/kg). Data are shown as percentage of mice, (n=10). Each stage's definition is described in section 2.4.

Treatment		Stage of seizures		
Treatment	**Organophosphate**	**Stage 0**	**Stage 1-3**	**Stage 4-6**
Cromakalim 0	**Dichlorvos**	0	0	100
	Physostigmine	0	0	100
Cromakalim 0.1	**Dichlorvos**	10	10	80
	Physostigmine	10	20	70
Cromakalim 10	**Dichlorvos**	40	20	40
	Physostigmine	50	20	30
Cromakalim 30	**Dichlorvos**	80	10	10
	Physostigmine	80	10	10
Glibenclamide 1	**Dichlorvos**	0	0	100
	Physostigmine	0	0	100
Cromakalim 30 + Glibenclamide 1	**Dichlorvos**	0	10	90
	Physostigmine	10	10	80

Also, cromakalim at doses of 0.1 µg/kg (P<0.01), 10 µg/kg (P<0.001), and 30 µg/kg (P<0.001) significantly increased the onset of death after both OP and carbamate administration (Figure 2).

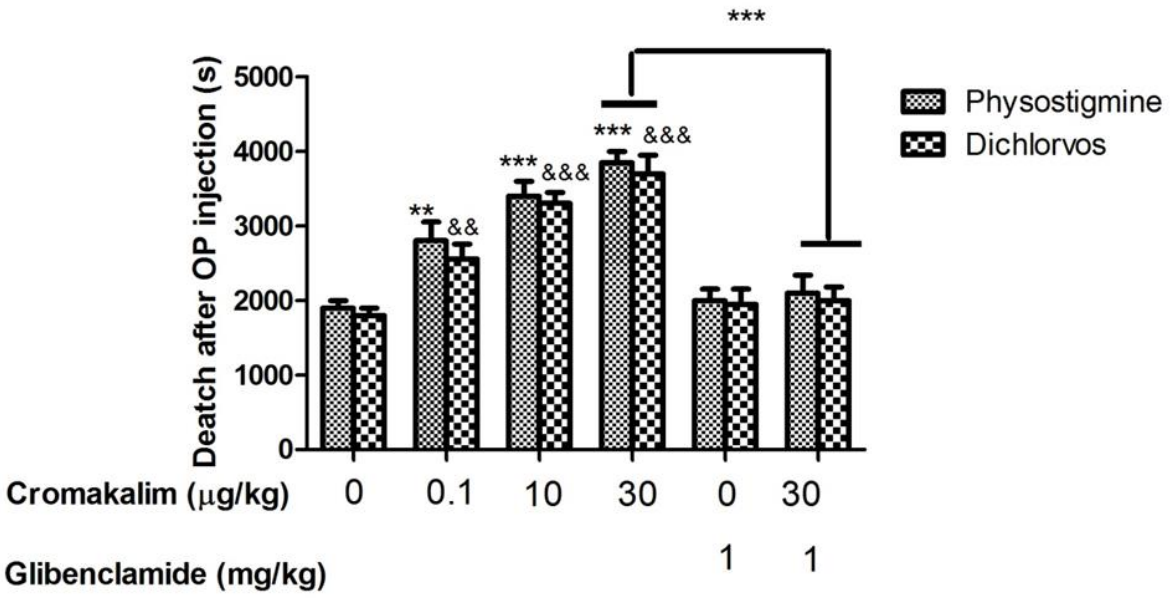

Figure 2. Effect of different doses of cromakalim (0.1, 10, and 30 µg/kg) and glibenclamide (1 mg/kg) on the latency of death after dichlorvos (50 mg/kg) and physostigmine (2 mg/kg) injection

Effect of cromakalim (30 µg/kg) and glibenclamide (1 mg/kg) co-administration on the onset of seizure in mice. Values are shown as mean+S.E.M. of 10 animals. $^{\&\&}P$<0.01, $^{\&\&\&}P$<0.001 compared to dichlorvos-injected group (control). $^{**}P$<0.01 and $^{***}P$<0.001 compared to physostigmine-injected group (control). $^{***}P$<0.001 compared to cromakalim (30 µg/kg) + glibenclamide (1 mg/kg) -administrated group.

Effect of cromakalim on the mortality induced by dichlorvos and physostigmine

Figure 3 shows that cromakalim administration reduces the percentage of mortality induced by dichlorvos and physostigmine. Cromakalim at doses of 0.1, 10, and 30 µg/kg decreased the mortality percentage compared to control group, which received dichlorvos and physostigmine.

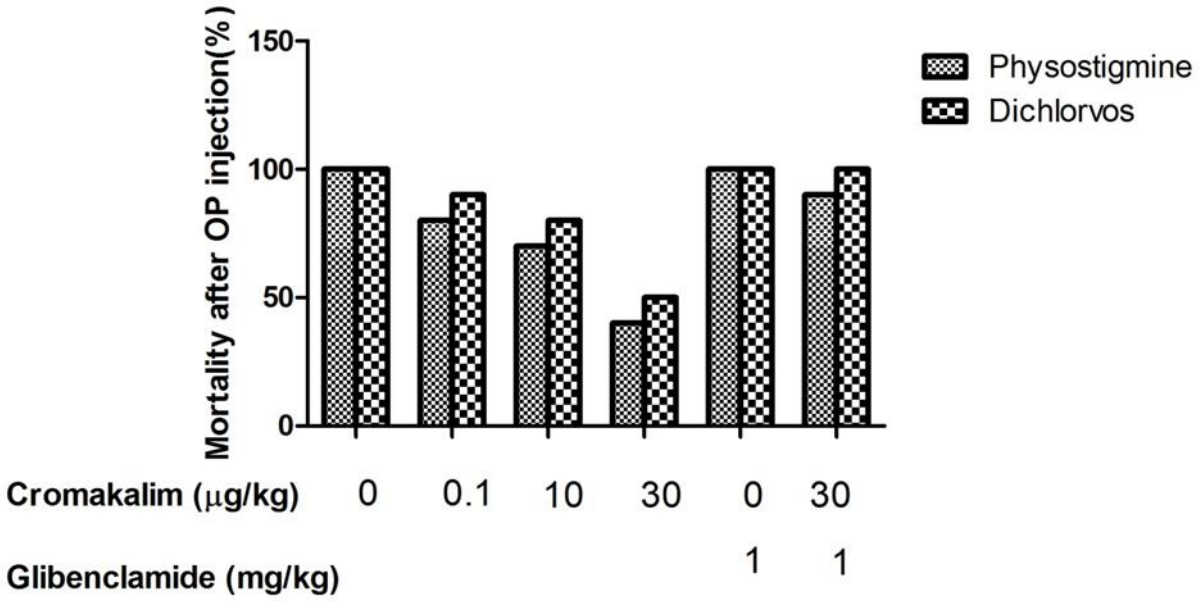

Figure 3. Effect of different doses of cromakalim (0.1, 10, and 30 µg/kg) and glibenclamide (1 mg/kg) on the mortality rate after dichlorvos (50 mg/kg) and physostigmine (2 mg/kg) injection

Effect of cromakalim (30 µg/kg) and glibenclamide (1 mg/kg) co-administration on the mortality rate in mice. Data are shown as percentage of mice (n = 10).

Effect of cromakalim on the seizure stages after dichlorvos and physostigmine administration

Demonstrating in Table 1, cromakalim at doses of 0.1, 10, and 30 µg/kg reversed the proconvulsant effect of both dichlorvos and physostigmine in mice. This anticonvulsant effect of the potassium channel opener, cromakalim shows a dose-dependent pattern, since by increasing the dose of this agent the percentage of stage 0 and stage 1-3 raised compared to the control group (Table 1).

Effect of glibenclamide on the anticonvulsant effect of cromakalim

Administration of glibenclamide (1 mg/kg, i.p.) 5 minutes before cromakalim injection (30 µg/kg) inhibited the anticonvulsant effect of cromakalim on OP- and carbamate-induced seizure (Table 1). It also decreased the latency time of clonic seizure (P<0.001) (Figure 1) as well as death time after injection (P<0.001) (Figure 2). Furthermore, glibenclamide increased the mortality rate (Figure 3).

Discussion

Dichlorvos and physostigmine cause colonic and tonic seizure and ultimately death in 100% of animals. After intraperitoneal injection of dichlorvos or physostigmine, some degree of tremor and excessive activity appear and then the symptoms become more severe and cause death over the time. Seizure is one of the toxic effects that happens following being poisoned with OPs and carbamates such as dichlorvos and physostigmine (18,19). These substances induce their effects by inhibiting the AChE in the nervous system. Depending on the level of AChE inhibition, cholinergic activation may cause hyperactivity of excitable tissues, fasciculations, seizures, convulsions, coma, and death (20). It has been suggested that systemic application of sub-lethal doses of AChE inhibitors (AChEIs) may result in seizures, convulsions, and central nervous system (CNS) lesions (21). These findings corroborate our results, which showed that dichlorvos at dose of 50 mg/kg as well as physostigmine at dose of 2 mg/kg cause convulsion and death in all animals.

Based on intervention with cholinergic system by these chemicals, past studies suggested using atropine, oximes, and benzodiazepines to control this kind of seizure. However, drugs typically used against epilepsy in the hospital are ineffective against OP/carbamates intoxication (6). For example, atropine improves only a few toxicity symptoms and shows severe adverse effects (22). Oximes permeate weakly through the blood-brain barrier (23), and they cannot treat the CNS toxicity, effectively. Benzodiazepines are likely to depress brainstem respiratory and circulatory centers (7). In this regard, conducting research to discover new anti-

epileptic drugs, which can inhibit the seizure induced by OPs and carbamates with favorable pharmacologic properties and side effect profile is unquestionable.

In our study, cromakalim, a potassium channel opener, reduced the rate of both seizure and mortality. The onset of seizure and death after OP/carbamate administration was diminished by cromakalim pretreatment and this is in agreement with previous data, which showed that diazoxide as an ATP-sensitive potassium channels opener exerted anticonvulsant activity against dichlorvos-induced seizure (5). Also, in the present study, glibenclamide as an ATP-sensitive potassium channels blocker, reversed the anticonvulsant effect of cromakalim.

Potassium (K^+) channels are a large family of ion channels. Among the different kinds of K^+ channels, ATP-sensitive K^+ (K_{ATP}) channels are involved in numerous physiological functions (8). K_{ATP} channels are located pre- and post-synaptically in many brain areas and their function is controlled by the metabolic condition of the neuron. They open and close in reply to the alterations in intracellular ATP/ADP relations. Low ATP degree opens these channels, leading to K^+ efflux and cell hyperpolarization (24). The hyperpolarization induced by K_{ATP} channels opening inhibits the action potential firing and prevents hyper-synchronous neuronal discharges which occur during seizure. In this regard, it has been shown that K_{ATP} channels play a significant role in regulation of seizure threshold in several *in vitro* and *in vivo* models (13,14,25). Also, K_{ATP} channel openers have been reported to decrease excitability in CA3 hippocampal cells (26), and show antiepileptic effects in a model of drug-induced epilepsy (27). Molecular studies have shown that functional K_{ATP} channels are octameric complexes consisting of four inward rectifier K^+ channel sub-units (Kir 6.1 or Kir 6.2) and four sulfonylurea receptor sub-units (SUR1, SUR2A or SUR2B) (11). Mice which lack expression of either the SUR1 gene or the Kir 6.1 gene are susceptible to seizures (28). Moreover, it has been shown that mice with deficiencies in a subunit of K_{ATP} channels (Kir $6.2^{-/-}$ mice) are vulnerable to generalized seizure (14). Also, it has been recently reported that K_{ATP} channel openers such as cromakalim and diazoxide increase the clonic seizures induced by pentylenetetrazol (PTZ) in mice (9,29).

Altogether, the role of potassium channel openers in the seizure has been shown in several clinical and animal studies. Nevertheless, the effect of these agents on the seizure induced by OPs and carbamates has not been reported yet. Therefore, we studied this matter, and our results revealed that cromakalim (K_{ATP} channel opener) at doses of 0.1, 10, and 30 µg/kg could reverse the convulsion and death following OPs/carbamates administration. Moreover, our data revealed that anti-epileptic effect of cromakalim could be reversed by glibenclamide (a K^+ channel blocker). These data confirm the role of potassium channels in mediating the proconvulsant effect of OPs and carbamates. These findings make it possible to introduce new aspect with specific targets to manage patients who suffer from OPs or carbamates toxicity.

In summary, this study shows for the first time that cromakalim (K_{ATP} channel opener) diminished the seizure induced by dichlorvos and physostigmine in mice. We also suggest using new aspect with specific targets to manage seizures from OPs/carbamates toxicity, although further investigation is needed to elucidate the efficacy of these agents in AChE inhibition-induced seizure.

Acknowledgment

This work was supported by a grant (96002757) from Iran National Science Foundation (INSF). The authors would be thankful to Dr. S. Ejtemaei-Mehr for his helpful comments on this manuscript.

References

1. Cocker J, Mason H, Garfitt S, Jones K. Biological monitoring of exposure to organophosphate pesticides. Toxicol Lett 2002;134:97-103.
2. Millard CB, Broomfield CA. Anticholinesterases: medical applications of neurochemical principles. J Neurochem 1995;64:1909-18.
3. Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect 1990;87:245-54.
4. Cable GG, Doherty S. Acute carbamate and organochlorine toxicity causing convulsions in an agricultural pilot: a case report. Aviat Space Environ Med 1999;70:68-72.
5. Jazayeri A, Zolfaghari S, Ostadhadi S. Anticonvulsant Effect of Diazoxide against Dichlorvos-Induced Seizures in Mice. ScientificWorldJournal 2013;2013:697305.
6. Lallement G, Dorandeu F, Filliat P, Carpentier P, Baille V, Blanchet G. Medical management of organophosphate-induced seizures. J Physiol Paris 1998;92:369-73.
7. Munro NB, Watson AP, Ambrose KR, Griffin GD. Treating exposure to chemical warfare agents:

implications for health care providers and community emergency planning. Environ Health Perspect 1990;89:205-15.
8. Yamada K, Inagaki N. Neuroprotection by K _{ATP} channels. J Mol Cell Cardiol 2005;38:945-9.
9. Shafaroodi H, Asadi S, Sadeghipour H, Ghasemi M, Ebrahimi F, Tavakoli S, et al. Role of ATP-sensitive potassium channels in the biphasic effects of morphine on pentylenetetrazole-induced seizure threshold in mice. Epil Res 2007;75:63-9.
10. Ghasemi M, Shafaroodi H, Karimollah AR, Gholipour T, Nezami BG, Ebrahimi F, et al. ATP-sensitive potassium channels contribute to the time-dependent alteration in the pentylenetetrazole-induced seizure threshold in diabetic mice. Seizure 2010;19:53-8.
11. Wickenden AD. Potassium channels as anti-epileptic drug targets. Neuropharmacology 2002;43:1055-60.
12. Porter RJ, Rogawski MA. New antiepileptic drugs: from serendipity to rational discovery. Epilepsia 1992;33:S1-6.
13. Narita M, Suzuki T, Misawa M, Nagase H, Nabeshima A, Ashizawa T, et al. Role of central ATP-sensitive potassium channels in the analgesic effect and spinal noradrenaline turnover-enhancing effect of intracerebroventricularly injected morphine in mice. Brain Res 1992;596:209-14.
14. Yamada K, Ji JJ, Yuan H, Miki T, Miki T, Sato S, Horimoto N, et al. Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. Science 2001;292:1543-6.
15. Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat Genet 1998;18:25-2.
16. Dekundy A, Kaminski RM, Turski WA. Dizocilpine improves beneficial effects of cholinergic antagonists in anticholinesterase-treated mice. Toxicol Sci 2003;72:289-95.
17. McLean M, Gupta R, Dettbarn W, Wamil AW. Prophylactic and therapeutic efficacy of memantine against seizures produced by soman in the rat. Toxicol Appl Pharmacol 1992;112:95-103.
18. Tattersall J. Seizure activity post organophosphate exposure. Front Biosci 2009;14:3688-711.
19. Dekundy A, Kaminski RM, Zielinska E, Turski WA. NMDA antagonists exert distinct effects in experimental organophosphate or carbamate poisoning in mice. Toxicol Appl Pharmacol 2007;219:114-21.
20. Milatovic D, Gupta RC, Aschner M. Anticholinesterase toxicity and oxidative stress. Sci World J 2006;6:295-310.,
21. Sparenborg S, Brennecke L, Jaax NK, Braitman DJ. Dizocilpine (MK-801) arrests< i> status epilepticus</i> and prevents brain damage induced by soman. Neuropharmacology 1992;31:357-68.
22. Bowden CA, Krenzelok EP. Clinical applications of commonly used contemporary antidotes. Drug Saf 1997;16:9-47.
23. Clement JG. Central activity of acetylcholinesterase oxime reactivators. Toxicol Appl Pharmacol 1992;112:104-9.
24. De Weille JR, Lazdunski M. Regulation of the ATP-sensitive potassium channel. Ion Channels 1990;2:205-22.
25. Katsumori H, Ito Y, Higashida H, Hashii M, Minabe Y. Anti-and proconvulsive actions of levcromakalim, an opener of ATP-sensitive K< sup>+</sup> channel, in the model of hippocampus-generating partial seizures in rats. Eur J Pharmacol 1996;311:37-44.
26. Alzheimer C, Ten Bruggencate G. Actions of BRL 34915 (Cromakalim) upon convulsive discharges in guinea pig hippocampal slices. Naunyn Schmiedebergs Arch Pharmacol 1988;337:429-34.
27. Gandolfo G, Gottesmann C, Bidard JN, Lazdunski M. Subtypes of K+ channels differentiated by the effect of K+ channel openers upon K+ channel blocker-induced seizures. Brain Res 1989;495:189-92.
28. Ben-Ari Y, Cossart R. Kainate, a double agent that generates seizures: Two decades of progress. Trends Neurosci 2000;23:580-7.
29. Niaki SEA, Shafaroodi H, Ghasemi M, Shakiba B, Fakhimi A, Dehpour AR. Mouth breathing increases the pentylenetetrazole-induced seizure threshold in mice: A role for ATP-sensitive potassium channels. Epil Behav 2008;13:284-289.