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Research article

Anticonvulsant effects of ivermectin on pentylenetetrazole- and maximal electroshock-induced seizures in mice: the role of GABAergic system and K_{ATP} channels

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HIGHLIGHTS

- Ivermectin exerts anticonvulsant effects on PTZ-induced clonic seizures.
- Ivermectin prevents MES-induced tonic-clonic seizures in mice.
- Ivermectin has the most anticonvulsant effects in doses of 1 and 5 mg/kg in mice.
- These anticonvulsant effects may be mediated through the GABAergic system.
- ATP-sensitive potassium channels could play a role in these anti-seizure effects.

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ABSTRACT

Introduction: Ivermectin (IVM) is an antiparasitic medicine that exerts its function through glutamate-gated chloride channels and $GABA_A$ receptors predominantly. There is paucity of information on anti-seizure activity of IVM. Moreover, the probable pharmacological mechanisms underlying this phenomenon have not been identified.

Materials and methods: In this study, pentylenetetrazole (PTZ)-induced clonic seizures and maximal electroshock (MES)-induced tonic-clonic seizure models, respectively in mice was utilized to inquire whether IVM could alter clonic seizure threshold (CST) and seizure susceptibility. To assess the underlying mechanism behind the anti-seizure activity of IVM, we used positive and negative allosteric modulators of $GABA_A$ (diazepam and flumazenil, respectively) as well as K_{ATP} channel opener and closer (cromakalim and glibenclamide, respectively). Data are provided as mean ± S.E.M. After the performance of the variance homogeneity test, a one-way and two-way analysis of variance was used. Fisher's exact test was performed in case of MES. P-value less than 0.05 considered statistically significant.

Results: and Discussion: Our data showed that IVM (0.5, 1, 5, and 10 mg/kg, i.p.) increased CST. Furthermore, flumazenil 0.25 mg/kg, i.p. and glibenclamide 1 mg/kg, i.p., could inhibit the anticonvulsant effects of IVM. Supplementary, an ineffective dose of diazepam 0.02 mg/kg, i.p. or cromakalim 10 μg/kg, i.p. were able to enhance the anticonvulsant effects of IVM. Besides, we figure out that the IVM (1 and 5 mg/kg, i.p.) could delay the onset of first clonic seizure and also might decrease the frequency of clonic seizures induced by PTZ (85 mg/kg, i.p.). Finally, IVM could prevent the incidence and death in MES-induced tonic-clonic seizures.

Conclusion: Based on the obtained results, it can be concluded that IVM may exert anticonvulsant effects against PTZ- and MES-induced seizures in mice that might be mediated by $GABA_A$ receptors and K_{ATP} channels.

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1. Introduction

Ivermectin (IVM), a member of the avermectin family [1], discovered in 1970. IVM is derived from avermectin B1, comprised of 22,23-dihydro-avermectin B1a and B1b in a ratio of 4 to 1 [2,3]. IVM is a highly efficient medication for treating many metazoan parasitic infections in invertebrates and has been known for its antiparasitic features [4]. IVM is considered a safe human medicine, especially in the treatment of onchocerciasis [5]. Several mechanisms of action are considered for IVM. The primary mechanism of action is related to the glutamate-gated chloride channels [6, 7].

Furthermore, the other possible pharmacological mechanism of IVM is the contribution of the gamma-aminobutyric acid type-A ($GABA_A$) receptor to its effect and thus it has been suggested that avermectin B1a acts on a modulatory binding site of benzodiazepine receptor and could stimulate it [8, 9]. In other word, IVM acts as a positive allosteric modulator of the $GABA_A$ receptor [10]. It has been well established that GABAergic system, and specially $GABA_A$ receptors which has been considered the main inhibitory neurotransmitter in the central nervous system (CNS), involved in modulating seizure threshold [11, 12, 13, 14, 15]. Benzodiazepines such as diazepam are considered a positive allosteric GABA receptors which raise the synaptic transmission of GABA [16] and increase the chloride ions current through the membrane of neurons causing hyperpolarization and decreasing excitability, which may be blocked by flumazenil as a negative allosteric modulator of $GABA_A$ receptor [17, 18, 19].

Besides the antiparasitic features of IVM, in our previous studies, we demonstrated that the GABAergic system is involved in the anti-inflammatory effects of IVM using skin flap model as well as colitis-induced by acetic acid in rats (0.05 and 0.5 mg/kg, respectively) [20, 21]. Moreover, some antiepileptic and anxiolytic attributes have been reported for IVM in numerous animal models [22, 23, 24, 25] and in clinical trials [26, 27, 28]. However, the effective doses of IVM with anticonvulsant effect at doses close to therapeutic doses have not been identified. Moreover, there are no studies, to the best of our knowledge, to clarify the pharmacological mechanisms involved in its anti-seizure effects.

ATP-sensitive potassium (K_{ATP}) channels are one of the most significant groups of ion channels [29], which are sensitive to changes in the ATP/ADP ratio as well as intracellular adenosine triphosphate levels, coupling the electrical activity of cells to their metabolic states and has involvement in several physiological functions [30, 31]. It has shown that K_{ATP} channels in the reticulata neurons may protect against energy-consuming generalized seizures by the earlier response to hypoxia [32]. Although it has been indicated that IVM modulates G protein-coupled inwardly-rectifying potassium channels [33, 34], there is no evidence to indicate whether K_{ATP} channels, contribute to the IVM's mode of action. Consequently, using K_{ATP} channels modulators, including cromakalim as a K_{ATP} channels opener and also glibenclamide as a K_{ATP} channels closer, we decided to evaluate the conceivable impact of these channels on the anti-seizure properties of IVM.

It should be considered that experimental research on seizures has been performed mainly through chemical or electrical means to induce seizures in normal mice [35, 36]. Drugs with anticonvulsive or pro-convulsive actions are signified by modifying the onset and severity of PTZ-induced seizures. PTZ is regarded as a non-competitive GABAA receptor antagonist [37, 38, 39]. There are intravenous (i.v.) and intraperitoneal (i.p.) injection of PTZ models in our current work, which have been utilized widely as the lab assessments to evaluate the anti-seizure effects of medication and to develop new antiepileptic therapies [40, 41, 42, 43, 44]. Similarly, the maximal electroshock-induced tonic-clonic seizure is the most severe electroshock convulsive response induced by supramaximal electrical stimulation currents. In fact, MES model that developed a few decades ago [45], is may be capable and accredited preclinical tests that forestall the drug's effectiveness against generalized seizures. Indeed, drugs that inhibit the tonic hindlimb extension (THLE) part of MES are effective anti-seizure medication against generalized tonic-clonic seizures [36, 37, 46, 47, 48].

As a result, we evaluate the efficacy of IVM on seizure threshold in mice models of clonic seizures induced by PTZ, as well as the potential involvement of K_{ATP} channels and the GABAergic system in these effects, in this study. Finally, we looked at the anticonvulsant impact of IVM on MES-induced tonic-clonic seizures.

2. Material and methods

2.1. Chemicals and route of administration

The drugs and chemicals used are as follows: Pentylenetetrazole, cromakalim, glibenclamide (Sigma, Bristol, UK), Ivermectin was received as a gift from Gilaranco (Rasht, Iran), diazepam (Kimidaru, Tehran, Iran), and flumazenil (Hameln Pharma Plus GmBH, Hamelin, Germany). DMSO 1% was utilized to dissolve IVM and glibenclamide. In 0.9% normal saline cromakalim, diazepam, and flumazenil were dissolved. All drugs and vehicles were administered i.p., in a constant volume of 10 ml/kg body weight of each mouse.

2.2. Animals

In this work, adult male NMRI mice weighing 25 ± 5 g were procured from the Pharmacology Department, Tehran University of Medical Sciences (TUMS), Tehran, Iran. Mice were kept in cages (36 * 22 * 14 cm) in groups of 8 with unrestricted access to water and food unless injected or tested. All experiments were carried out between 10:00 a.m. and 2:00 p.m. in a room with suitable conditions, including a 12-hour regular light/dark cycle as well as a temperature of 22 °C and humidity of 55 percent.

All the study protocols were authorized by the committee of animal ethics and experiments at TUMS, Tehran, Iran (IR.TUMS.MEDICINE.REC.1400.437).

2.3. Investigation of anticonvulsant properties of ivermectin

As mentioned before, three different animal models of seizure induction, including i.v. and i.p. PTZ-induced clonic seizures as well as MES-induced tonic-clonic seizure were used to evaluate the anticonvulsant effects of IVM.

2.3.1. Evaluation of clonic seizure threshold induced by intravenous pentylenetetrazole

In order to measure CST an infusion pump (Harvard, USA) was used to infuse the PTZ solution (0.5 % in saline) at a constant rate of 0.5 mL/min into the tail vein of the freely moving mice. The tape was used to keep the needle gauge 30 in the tail vein. The infusion was paused immediately after forelimb clonus, followed by complete body clonus and loss of balance. Moreover, the least required dosage of PTZ (measured in mg/kg of each mouse) to induce seizure has been calculated as CST using the formula below:

$$\text{PTZ}\left(\frac{\text{mg}}{\text{kg}}\right)=\frac{\text{Infusion Duration (s)}*\text{ Infusion Rate }\left(\frac{\text{ml}}{\text{min}}\right)*\text{ PTZ Concentration }\left(\frac{\text{mg}}{\text{ml}}\right)*1000\left(\frac{\text{g}}{\text{kg}}\right)}{\text{Weight (g)}*60\left(\frac{\text{s}}{\text{min}}\right)}$$

2.3.2. Seizures induced by intraperitoneal pentylenetetrazole model

Three parameters were evaluated using i.p. PTZ injection at a dosage of 85 mg/kg, which is considered CD_{97} for clonic seizures in current research. The first is the time it takes for clonic seizures to start, the second is the number of seizures, and the third is the frequency of death after the seizures within 30 min. Finally, the positive control was treated with diazepam 0.5 mg/kg.

2.3.3. Tonic-clonic seizure induced by maximal electroshock

Tonic-clonic seizures in mice were induced using an electro-convulsiometer by administering electroshock at 60 Hz and 50 mA of intermittent current for 1 s through electrodes affixed to the mouse ears. The incidence of THLE (180° extension of mouse hindlimbs relative to the body axis) was used as a criterion for seizure activity. In other word, the most common endpoint for anticonvulsant drug activity in MES is the inhibition of THLE.

2.4. Experimental protocol

Experiment 1: In the first experiment, we assessed the anti-seizure action of IVM at doses close to therapeutic levels and determined the optimal dose by comparing the effects of different IVM doses on the i.v. PTZ-induced clonic seizure threshold. Multiple doses of IVM of its vehicle were injected 30 min before PTZ infusion. The ineffective and effective IVM doses were determined in this experiment. Furthermore, a time course study was also conducted, with intervals of 30 min, 15 min, and 45 min between IVM and PTZ injection being studied. Times utilized in this study were chosen in accordance with previous research [49, 50].

Experiment 2: In order to discern the feasible involvement of GABA receptors in anticonvulsant activities of IVM, the $GABA_A$ receptor positive allosteric, diazepam at the ineffective dose of (0.02 mg/kg, i.p.), which in our previous studies considered as an ineffective dose, were injected 5 min before IVM (0.2 mg/kg) and 35 min before PTZ infusion. Time intervals and dose selections was based on our previous studies [51, 52, 53].

Experiment 3: Flumazenil, at an ineffective dose of 0.25 mg/kg had been chosen to indicate whether inhibition of Cl^- channels of the GABAergic pathway could have an effect on the anticonvulsant effects of IVM. Flumazenil was injected 20 min prior to IVM (5 mg/kg i.p.). Alongside, flumazenil was also given i.p. 15 minutes before diazepam (0.02 mg/kg) and 20 min before IVM (0.2 mg/kg). The mentioned dose and time intervals were based on our previous studies [51, 53, 54].

Experiment 4: We used glibenclamide (1 mg/kg, i.p.) as a K_{ATP} channel blocker 30 min before injecting IVM (5 mg/kg) and 60 min before the measurement of CST in order to investigate possible involvement of K_{ATP} channels in CST modulation by IVM. This time interval was based on previous studies done in our lab [55, 56, 57, 58]. It should be noted that in our prior experiments, the influence of various

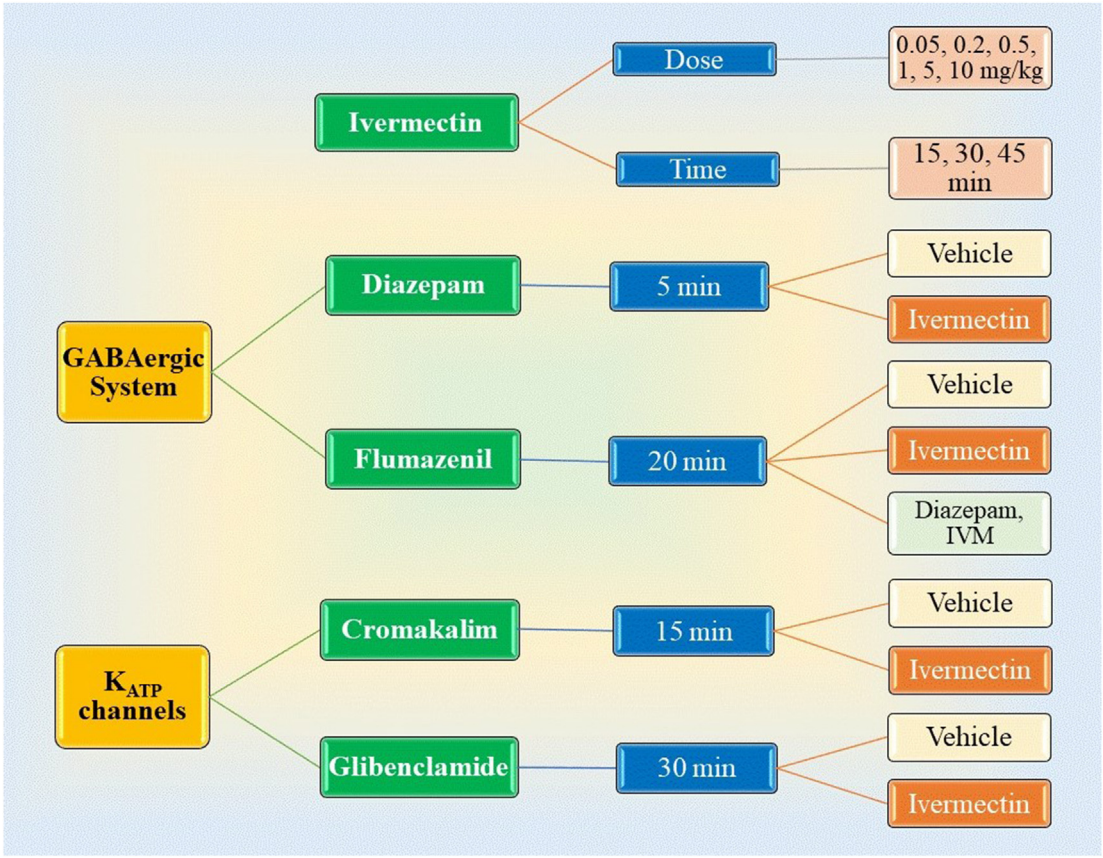

Figure 1. Schematic diagram for displaying presentation of medication administration prior to PTZ-induced clonic seizures in various experimental groups of mice.

glibenclamide dosages on CST was studied. It was determined that the maximum ineffective dose of glibenclamide for changing CST is 1 mg/kg. Moreover, this point should be mentioned that glibenclamide at this dose can't significantly alter the plasma glucose level based on the previous studies [58].

Experiment 5: We administered cromakalim (at an ineffective dose of 10 μg/kg), which has been considered the K_{ATP} channel opener, 15 min before administering the ineffective dose of IVM (0.2 mg/kg, i.p.) in order. Also, 30 min after the IVM injection, the seizure threshold was measured. It is worth noting that in our prior experiments the impact of different cromakalim dosages on CST as well as its time course was studied. It was shown that the maximal ineffective dose of cromakalim for altering CST is 10 μg/kg [59]. In this regards this dose was injected 15 min prior IVM administration in our study [55, 56, 57, 58]. The experimental protocol of our study was shown in Figure 1.

Experiment 6: In the second seizure animal model, the frequency of seizure incidence as well as the time it takes for seizures to start (latency) and the death in the clonic seizures induced by pentylenetetrazole (85 mg/kg, i.p.) were examined and recorded.

Experiment 7: In the third seizure model in mice, the impact of IVM at the various doses on MES-induced tonic seizures were perused. Incidence of THLE as well as death was compared between groups.

2.5. Statistical analysis

Data are presented as mean ± standard error of the mean (S.E.M.), and data analysis was done using SPSS (Version 26) with the help of GraphPad Prism (version 9). After the performance of the variance homogeneity test, a one-way and two-way analysis of variance (ANOVAs) was used, followed by post hoc Tukey's tests. However, in case of time latencies for the onset of the first clonic seizure, data were presented as medians (with 95% confidence intervals) and the Mann-Whitney U test was conducted to do a non-parametric analysis based on median values. Fisher's exact test was utilized to analyze the data in MES paradigm. In all experiments, a P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Evaluation of the anticonvulsant effect of ivermectin

3.1.1. Effect of ivermectin on CST in mice induced by i.v. PTZ

The effects of acute i.p. injection of IVM (0.05, 0.2, 0.5, 1, 5, and 10 mg/kg) in mice on the CST are shown in Figure 2A. IVM was administered 30 min before PTZ. IVM at the doses of 0.5 mg/kg, 1 mg/kg, 5 mg/kg, and 10 mg/kg increase the CST considerably ($P < 0.05$, $P < 0.001$, $P < 0.0001$, and $P < 0.01$, respectively). However, IVM (0.05 mg/kg and 0.2 mg/kg) had no significant anticonvulsive effects ($P > 0.05$).

3.1.2. Time course study of the anticonvulsant effect of IVM on CST

The time course study of the anti-seizure activity of IVM (5 mg/kg, which was the most effective dose) is shown in Figure 2B. IVM (5 mg/kg) given 15 min before PTZ infusion had no effect on CST ($P > 0.05$). The identical dosage of IVM given 30 min before seizure induction considerably affected the CST (****$P < 0.0001$), whereas it had a smaller but still significant effect 45 min before the test (**$P < 0.01$). Compared to DMSO-treated mice (as a control group), the data reveal that IVM has an anti-convulsant effect with peak activity 30 min after treatment.

3.2. Effects of GABAergic receptor modulators on anticonvulsant effects of ivermectin

3.2.1. Effect of flumazenil on anticonvulsant properties of ivermectin

As it indicated in Figure 3A., the impact of acute i.p. injection of $GABA_A$ receptor antagonist flumazenil on PTZ-induced CST is evaluated. Based on the obtained results, the acute treatment of an ineffective dose

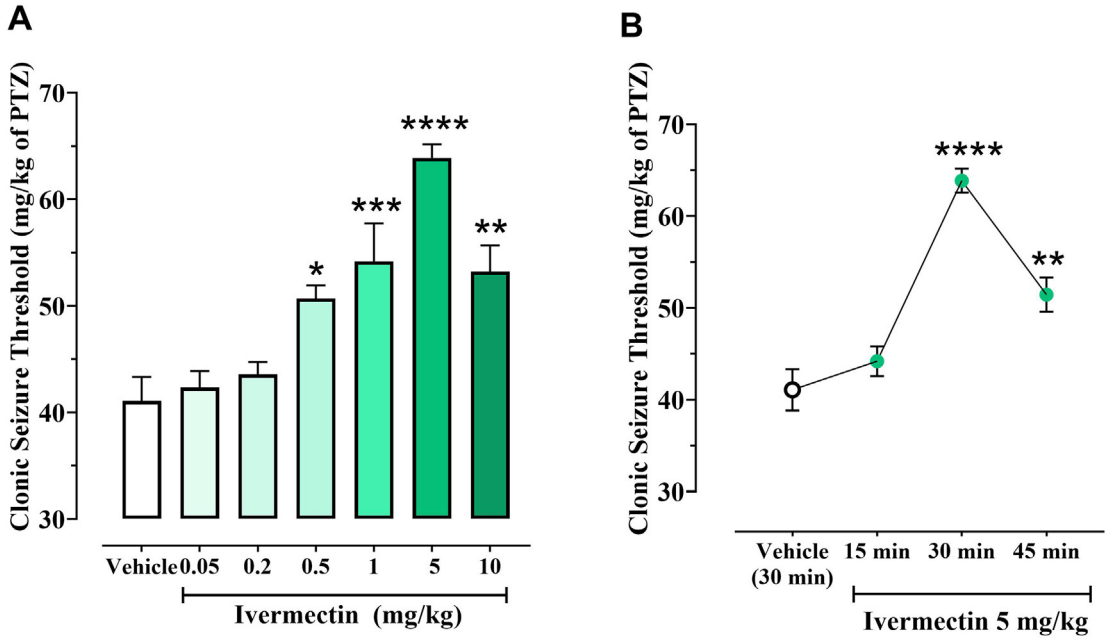

Figure 2. A) The effects of acute i.p. injection of ivermectin (0.05, 0.2, 0.5, 1, 5, and 10 mg/kg, i.p., 30 min before PTZ infusion) on the CST ($F (6, 49) = 15.22$, $P < 0.0001$, One-way ANOVA) B) The effect of ivermectin (5 mg/kg) treatment on CST in multiple time interval. Ivermectin (5 mg/kg) was given 15, 30, and 45 min before the PTZ injection (One way ANOVA, $P < 0.0001$, $F (3, 28) = 32.12$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$p < 0.0001$ as an indicator for comparing treated groups with vs. control (DMSO 1% in saline) group. Data are reported as mean ± SEM and each group contains 8 mice.

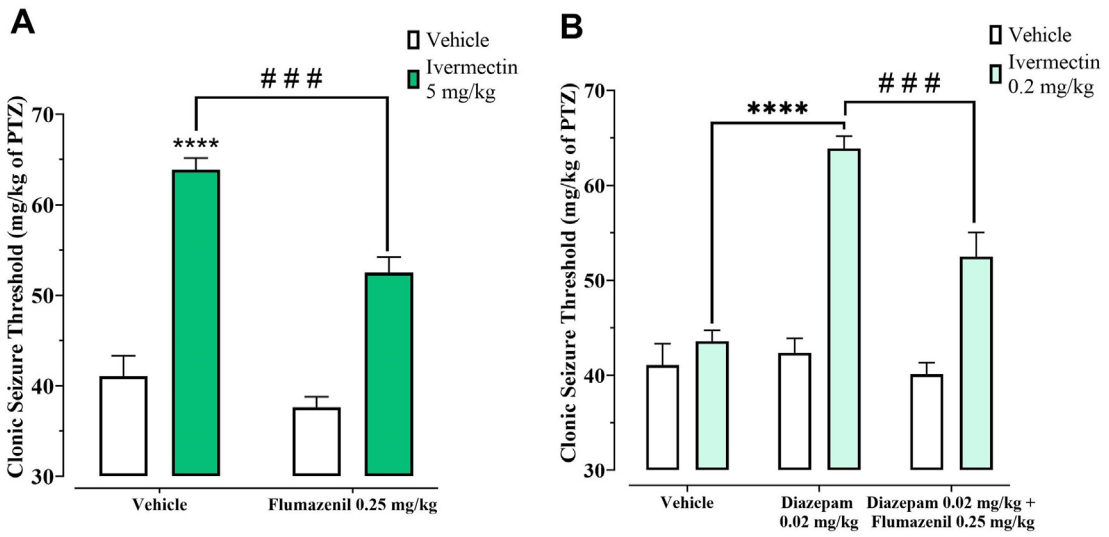

Figure 3. A) Pretreatment with an ineffective dose of flumazenil reduced significantly the anticonvulsant activity of ivermectin (IVM, 5 mg/kg, i.p.). ####$<$P $<$ 0.001 comparison between Saline/IVM versus flumazenil + IVM, ****P $<$ 0.0001 comparison between IVM (5 mg/kg) and DMSO-treated as a control group (Two-way ANOVA, F (3, 21) = 54.01, P $<$ 0.001). B) Effects of Diazepam (0.02 mg/kg, i.p.) on the ineffective dosage of ivermectin (IVM, 0.02 mg/kg, i.p.) and the combination IVM plus diazepam with flumazenil on the CST (Two-way ANOVA, F (4, 28) = 30.54, P $<$ 0.0001). ****P $<$ 0.0001 when comparing IVM/saline to IVM/Diazepam, and ###P $<$ 0.001 when comparing IV/Diazepam/vehicle to IVM/Diazepam/Flumazenil group. Data are reported as mean ± SEM and each group contains 8 mice.

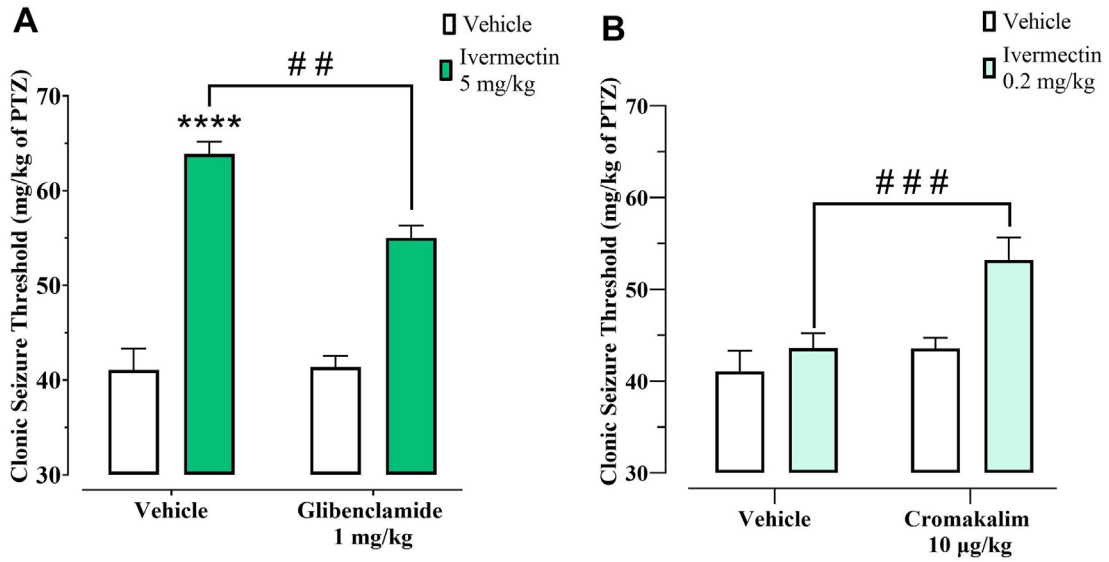

Figure 4. A) Pretreatment effects of glibenclamide (1 mg/kg, i.p.) or its vehicle (DMSO) on the anticonvulsant effects of ivermectin (IVM, 5 mg/kg, i.p.) on CST. Glibenclamide/IVM (5 mg/kg) versus DMSO/IVM (5 mg/kg) were compared with each other ##P $<$ 0.01 (Two-way ANOVA, F (3, 21) = 40.04, P $<$ 0.01). B) Effects of an ineffective dosage of cromakalim (10 µg/kg, i.p.) on ineffective dose of IVM (0.2 mg/kg, i.p.) on CST. When comparing the saline/IVM and Cromakalim/IVM groups, ###P $<$ 0.001 was found (Two-way ANOVA, F (3, 21) = 8.280, P $<$ 0.001). Data are reported as mean ± SEM and each group contains 8 mice.

of flumazenil (0.25 mg/kg, i.p.) 20 min before IVM reduced the anticonvulsant efficacy of IVM (5 mg/kg).

3.2.2. *Effects of flumazenil on anticonvulsant effects in mice*

Figure 3B depicts the effect of diazepam administration on anticonvulsant effects of IVM. Diazepam at a dose of 0.02 mg/kg or vehicle were administered 5 min before IVM (0.2 mg/kg). Diazepam (0.02 mg/kg) alone had no effect on CST while, diazepam (0.02 mg/kg) in combination with IVM (0.2 mg/kg) group, was elevated the CST considerably (****P $<$ 0.0001) compared to IVM-treated (0.2 mg/kg) mice. Taking the same path, it was also found that synergistic effects between IVM and diazepam on CST could be prevented by acute i.p. injection of flumazenil (P $<$ 0.001).

3.3. *Effects of K_{ATP} channel modifiers on the ivermectin anticonvulsant activities*

3.3.1. *Glibenclamide effect on anticonvulsant activity of ivermectin*

Figure 4A indicates that glibenclamide, reduces anticonvulsive effects of IVM (##P $<$ 0.01 and ****P $<$ 0.0001). It's important to note that at this dose, glibenclamide had no discernible trace on the clonic seizure threshold triggered by PTZ.

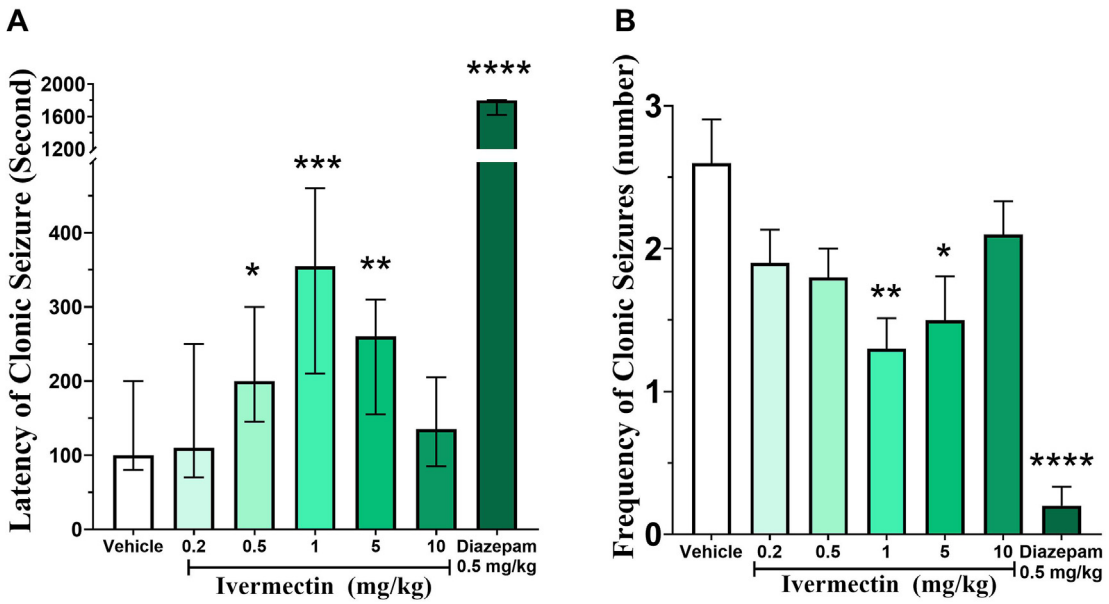

Figure 5. Each group contains 10 mice and diazepam (0.5 mg/kg, i.p) considered positive control group. A) Data are mentioned as medians (with 95% confidence intervals) and Mann-Whitney U test was executed. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 *versus* vehicle. B) The number of clonic convulsions in a 30-minute period was compared among different treatment and control groups. Data are reported as mean ± SEM. *P < 0.05, **P < 0.01, ****P < 0.0001 *versus* control group (One-way ANOVA, F (6, 63) = 10.02, P < 0.0001).

Table 1. Effects of IVM on the protection against death after i.p. injection of PTZ (85 mg/kg).

Groups	Survival (%)	P-value
Negative Control (DMSO 1% in Saline 0.9%)	40 %	-
Ivermectin 0.5 mg/kg	40 %	>0.999 (ns)
Ivermectin 0.5 mg/kg	60 %	0.3698 (ns)
Ivermectin 1 mg/kg	70 %	0.1698 (ns)
Ivermectin 5 mg/kg	60 %	0.3698 (ns)
Ivermectin 10 mg/kg	50%	>0.999 (ns)
Positive Control (Diazepam 0.5 mg/kg)	100 %	<0.01 (**)

Survival considered as percentage of mice that still survive after i.p. PTZ injection. Comparison was performed using Fisher's exact test. (ns) P > 0.05, *P < 0.05, **P < 0.01 when compared to negative control group (vehicle).

Table 2. Effects of ivermectin on the incidence of tonic-clonic seizure caused by MES in mice.

Groups	Protection (%)	P-value
Negative Control (DMSO 1% in Saline 0.9%)	6.67%	-
Ivermectin 0.5 mg/kg	40%	0.0801 (ns)
Ivermectin 1 mg/kg	53.33%	0.0142 (*)
Ivermectin 5 mg/kg	60%	0.0019 (**)
Ivermectin 10 mg/kg	46.67%	0.0352 (*)
Positive Control (Diazepam 0.5 mg/kg)	86.66%	<0.0001 (****)

Protection considered as percentage of preventing versus seizure incidence. Comparison was performed using Fisher's exact test. In fact, (ns) P > 0.05, *P < 0.05, **P < 0.01 and *P < 0.05 when comparing different group to negative control group (vehicle).

3.3.2. Cromakalim effect on anticonvulsant activity of ivermectin effect

As demonstrated in Figure 4B, administration of the selective K_{ATP} channel opener cromakalim (10 μg/kg i.p.) 15 min before injecting an ineffective dose of IVM (0.2 mg/kg i.p.) caused a considerable increase in CST (***P < 0.001). Cromakalim alone had no impact on CST at this dosage.

Table 3. Effects of IVM on the mortality caused by MES-induced tonic-clonic seizures in mice.

Groups	Survival (%)	P-value
Negative Control (DMSO 1% in Saline 0.9%)	46.67%	-
Ivermectin 0.5 mg/kg	80%	0.1281 (ns)
Ivermectin 1 mg/kg	100%	0.0022 (**)
Ivermectin 5 mg/kg	100%	0.0022 (**)
Ivermectin 10 mg/kg	73.33%	0.2635 (ns)
Positive Control (Diazepam 0.5 mg/kg)	100%	0.0022 (**)

Fisher's exact test compared groups to negative control group. (ns) P > 0.05, **P < 0.01 when comparing each group to vehicle.

3.4. Effects of ivermectin on seizures induced by intraperitoneal injection of PTZ in mice

IVM at the doses of 0.5, 1 and 5 mg/kg significantly increased the latency for the onset of first clonic seizure occurrence (Figure 5A), and decreased the frequency of clonic seizures during the 1800s (Figure 5B). However, no significant difference in mortality was observed between different IVM-treated groups (see Table 1).

3.5. Effect of ivermectin on maximal electroshock-induced seizure

In the maximal electroshock (MES)-induced tonic-clonic seizure model, IVM (1, 5 and 10 mg/kg) treatment could protect the mice against the development of tonic seizure, as indicated in Table 2. IVM (1 and 5 mg/kg) considerably prevents mice from mortality (Table 3). It should be mentioned that each group consisted of 15 mice and a positive control group was considered with diazepam (0.5 mg/kg, i.p.).

4. Discussion

In this study, we demonstrated that IVM has protective effects against both i.v. and i.p. pentylenetetrazole-induced clonic seizure as well as maximal electroshock-induced tonic-clonic seizure models in mice. PTZ-induced clonic seizure is a type of forebrain-controlled seizure in which

the activity of main forebrain epileptogenic areas increases [60, 61]. Based on our obtained results, IVM (0.5, 1, 5, and 10 mg/kg, i.p.) reduced seizure susceptibility induced by PTZ in our investigation, whereas lower dosages (0.05 and 0.2 mg/kg) had no significant influence. On the other hand, MES significantly impacts the brainstem's neuronal pathways that induce tonic-clonic convulsive and THLE [47, 48]. Based on the result of our study, IVM (1 and 5 mg/kg) exerts anticonvulsant effects against MES-induced tonic-clonic seizure and inhibits THLE. It's worth noting that, despite the i.p. injection PTZ model, where IVM at a dose of 1 mg/kg has the best impact, IVM at a dose of 5 mg/kg has the best effect in the i.v. PTZ as well as MES models.

In other studies, anticonvulsant and anxiolytic effects of doramectin and ivermectin have been shown [22, 23, 24, 25]. For example, doramectin in doses (100, 300, and 1000 μg/kg, subcutaneous) has anxiolytic properties in mice [23]. Also, in the model of toxicity caused by monomethyl hydrazine (MMH), a common rocket propellant, IVM showed protective effects. IVM (15, 10, 5 mg/kg) prevented death but had no effect on the incidence of seizures [62]. Furthermore, in other seizure animal models, after the i.p. injection of avermectin B with a dose of 30–50 mg/kg and 10–20 mg/kg, seizures induced by sound and cefazoline were controlled, respectively [24]. In another study, the anticonvulsant effect of IVM was investigated in lidocaine- and strychnine-induced seizure models. In seizures induced by lidocaine and strychnine, the effective dose was 2.44 mg/kg and 4.25 mg/kg, respectively [63].

The inhibitory effects of the GABAergic system in the CNS are well-known [64]. Considering the vital role of the GABAergic system in epilepsy, in this study using pharmacological interventions the possible involvement of $GABA_A$ receptors on the anticonvulsant effect of IVM has been investigated. In this regard, we used the positive and negative allosteric modulators of the $GABA_A$ receptor (diazepam and flumazenil, respectively) to investigate their effects on the anticonvulsant effect of IVM. In our study, diazepam has been demonstrated to increase the anticonvulsant effects of IVM. On the other hand, the anticonvulsant action of IVM was decreased by pretreatment of mice with flumazenil, indicating that the GABAergic system might be involved in the anti-seizure ability of IVM against PTZ-induced clonic convulsions. Interestingly, flumazenil was able to inhibit the synergistic effect between diazepam and IVM significantly.

In the previous studies, various derivatives of the avermectin family were synthesized and investigated in the PTZ-induced tonic seizure model. It was found that the derivatives showed anticonvulsant effect, bind to the alpha1beta3gamma2 subunit of the $GABA_A$ receptor with high affinity [22], which is in line with our results. Furthermore, in another study it has been revealed that, coadministration of diazepam (5 mg/kg) with IVM (10 mg/kg) prevented seizures and death induced by MMH in mice. Meanwhile, IVM in the mentioned dose could not have this effect [62]. Moreover, in another animal study, flumazenil prevents only some part of anticonvulsive effects of IVM in lidocaine- and strychnine-induced seizures in rats [63]. Furthermore, based on the in vitro experiments, it has been indicated that IVM interacts with benzodiazepine receptors in rat cortex and cerebellum [65]. In addition to the importance of molecular pathways investigation, from a clinical point of view the administration of IVM as an adjuvant therapy in epileptic patients suffering from parasitic diseases is also being investigated. In clinical studies, it has been determined that the coadministration of IVM in combination with drugs such as phenobarbital with GABAergic effects, can reduce the occurrence of seizures in these patients [26, 28, 66]. Therefore, it is very important to find drug interactions in order to make the treatment more effective, and additional studies in this field seem necessary.

K_{ATP} channels are inwardly-rectifying potassium channels broadly expressed throughout the body [67]. Adenine nucleotides regulate K_{ATP}, characteristically activated by decreasing ATP and increasing ADP levels. To put it another way, in various in vitro investigations, K_{ATP} channels have been found to perform a significant contribution to CST modulation in vitro as well as in vivo models [55, 56, 57, 68, 69, 70, 71, 72]. It, therefore, performs essential physiological functions by linking cellular metabolism to membrane excitability [73]. In fact, it has been revealed that coadministration of cromakalim as a K_{ATP} channel opener with many drugs including melatonin [55], levosimendan [56], zolpidem [57], and morphine [58] may potentiates their anticonvulsant effects. In the same direction, pretreatment of mice with glibenclamide as a K_{ATP} channel blocker might inhibit anticonvulsant effects of the mentioned drugs. Although IVM was shown to be a GIRK channel activator [33, 34, 74, 75], to our knowledge there was no evidence that IVM may interact with K_{ATP} channels until now.

For the first time, our findings showed a possible role for K_{ATP} channels in this phenomenon. We used glibenclamide and cromakalim, K_{ATP} channel blockers and openers in ineffective doses (1 mg/kg, i.p. and 10 μg/kg, i.p., respectively) to understand whether these channels are involved in the anticonvulsant effects of IVM. Based on the obtained results of our study, an ineffective dose of glibenclamide could barricade anticonvulsant effects of IVM. On the other hand, an ineffective dose of cromakalim augmented the mentioned effects. In other word, in the i.v. PTZ-induced clonic seizures paradigm, glibenclamide decreased the anti-seizure effect of IVM noticeably, whereas cromakalim amplified antiepileptic actions of IVM.

Sulfonylurea receptor 1, a subunit of GIRK channels is found in the cytoplasm of GABAergic neurons, where K_{ATP} channels regulate gamma-Aminobutyric acid release [76]. In fact, the presence of K_{ATP} channels in both pre-synaptic and post-synaptic hippocampus neurons affects GABA release and response, respectively [77, 78]. K_{ATP} channel activators have been shown to decrease GABAergic transmission by lowering GABA release and influencing the post-synaptic GABA response [19, 76, 79]. Consequently, it's plausible that anti-seizure activities of IVM are probably mediated by a synergistic interaction between GABAergic and K_{ATP} channels. Based on the results of pharmacological interventions in our study, at least part of the anticonvulsant effects of IVM in i.v. PTZ-induced clonic seizures can be influenced by $GABA_A$ receptor allosteric modulators as well as K_{ATP} channels activator and inhibitor.

5. Conclusion

In conclusion, our findings revealed anticonvulsant effects of ivermectin in animal models of inducing seizures, including clonic seizures induced by PTZ (i.v. infusion and i.p. injection) and MES-induced tonic-clonic seizures in mice. Furthermore, using pharmacological interventions, we found that both the GABAergic system as well as K_{ATP} channels have an interference in the anticonvulsant effects of ivermectin.

Declarations

Author contribution statement

Mohammad Amin Manavi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Razieh Mohammad Jafari: Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data.

Hamed Shafaroodi: Conceived and designed the experiments; Analyzed and interpreted the data.

Shahram Ejtemaei-Mehr; Mohammad Sharifzadeh: Contributed reagents, materials, analysis tools or data.

Ahmad Reza Dehpour: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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