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Antiepileptic effects of quinine in the pentylenetetrazole model of seizure

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ABSTRACT

Quinine, is an anti-malarial drug that specifically blocks connexin 36 (Cx36) at gap junction channels. Quinine has suppressed ictal epileptiform activity *in vitro* without decreasing neuronal excitability. Thus, we considered the possible anticonvulsant effects of quinine in the pentylenetetrazole (PTZ) model of seizure. Moreover, we studied the hypnotic effect and locomotor activity of quinine in mice. In the PTZ model, quinine at the dose of 60 mg/kg increased the latency of seizure. However, quinine at 40–60 mg/kg decreased the duration of seizure, dose dependently. In the potentiation of pentobarbitone sleep test, quinine significantly increased sleeping time and decreased latency to fall asleep at doses of 50 and 60 mg/kg in mice. Also, quinine decreased total locomotion in the present study. It can be concluded that quinine possesses anticonvulsant and hypnotic effects, which could contribute to the control of seizure.

1. Introduction

Epilepsy is one of the most common serious neurological conditions with an annual incidence of 50/100,000 per year.[1,2] Seizures are controlled in nearly 70% of patients with epilepsy, mostly through drug effects on membrane ion channels or on GABAergic or glutamatergic transmission. However, for the remaining 20–30%, with intractable seizures, recent advances in systemic antiepileptic drug (AED) development have had little impact. Refractory epilepsy is associated with considerable medical, social, and psychiatric morbidity and enormous financial cost. Thus, novel approaches to the treatment of these patients are needed.[3,4] Abnormal synchronization of neuronal discharges is of recognized critical importance in seizures; however, the mechanisms underlying this pathological synchrony remain uncertain. In this context, there is growing interest in electrotonic communication via gap junctions, and speculation, based largely on studies *in vitro* and on ex vivo brain tissue that gap junctions may be important in the generation and propagation of seizures. The pathogenesis of abnormal neuronal synchrony underlying seizures, formerly thought to be based mainly on the chemical synaptic transmission, now includes a role of gap junctional communication. This concept has been strengthened by evidence from several *in vitro* models, in which pharmacological manipulations of gap junctional communication predictably affect the generation of seizures, with blockers diminishing seizures, and enhancers increasing seizures.[5,6] Thus, it seems that gap junctions may represent a novel therapeutic target for the future. Gap junction channels of vertebrates are formed of a family of proteins known as connexins (Cx) that are expressed in an overlapping pattern of tissue distribution. Quinine, an anti-malarial drug, specifically blocks Cx36 and with lesser potency Cx50 in mammalian cells.[7] Cx36 is exclusively expressed in neurons, being the principal connexin in adult neurons and has been linked to other genetic markers of juvenile myoclonic epilepsy (JME).[8,9] In contrast, Cx50 is not expressed in the mammalian brain.[10] Quinine was reported to suppress ictal epileptiform activity *in vitro* without decreasing neuronal excitability.[11] Recently, quinine suppressed epileptiform activity by decreasing the amplitude and frequency of epileptiform spikes and by attenuating the epileptiform behavior in rats.[12] Moreover, it inhibited cortical spreading depression (SD) on rat neocortical slices *in vitro*.[13] In a previous study, we reported that carbenoxolone, a gap junction blocker according to the pentylenetetrazole (PTZ) model, had anticonvulsant effects. Also, carbenoxolone showed hypnotic and muscle relaxant effects in mice.[14] Thus, we set out to investigate, in the PTZ model, the anticonvulsant effects of another gap junction blocker, quinine; hypothesizing that if gap junction channels are important in seizure generation and/or propagation, they will reduce the frequency or severity of seizures and might suggest novel treatment strategies for seizure in humans. In the present study we also examined the pentobarbitone sleep test and the open field

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test at anticonvulsant doses of quinine. It is important to know that at anticonvulsant doses, quinine causes a hypnotic effect and could reduce locomotor activity. This led us to compare these behavioral results with other gap junction blockers.

2. Materials and methods

2.1. Chemicals

Quinine anhydrous hydrochloride was purchased from Fluka. PTZ and pentobarbitone were obtained from Sigma. Diazepam was purchased from Daru Pakhsh Pharmaceutical Co., Iran in injectable form. PTZ and pentobarbitone were dissolved in physiological saline solution and quinine was dissolved in 0.8% (v/v) Tween 80. All drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg.

2.2. Animals

Male BALB/c mice (25–30 g) were obtained from the Razi Institute (Karaj, Iran). The animals were housed in standard Plexiglas cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at $23 \pm 3\ ^{\circ}C$ with a 12-h light/dark cycle (light on from 6 a.m.). Experiments were carried out between 8 a.m. and 1 p.m. All animal experiments were carried out in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC) in such a way to minimize the number of animals and their suffering.

2.3. Anticonvulsant activity

2.3.1. Pentylenetetrazole seizure model

The mice were divided into seven groups of 10 animals each for a total of 70 mice. In five groups, the mice were given quinine at the doses of 20, 30, 40, 50, or 60 mg/kg 30 min before the administration of PTZ (90 mg/kg). Two groups were injected with diazepam, the positive control (0.5, 1 mg/kg) and one group, the control group, was injected with normal saline + Tween 80 (10 ml/kg) 30 min before the administration of PTZ (90 mg/kg).[15] Each animal was placed into an individual plastic cage for observation lasting 1 h. The onset of a general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded.[15]

2.4. Potentiation of sodium pentobarbitone sleep test

Quinine at doses of 20, 30, 40, 50, 60 mg/kg, diazepam at doses of 0.5 and 1 mg/kg, or normal saline + Tween 80 (10 ml/kg) injected i.p. into mice of each group, respectively. Thirty minutes after initial drug injection, each animal was injected with sodium pentobarbitone (30 mg/kg, i.p.). The sleeping time was noted by recording the interval between the loss of and regaining of righting reflex.[16]

2.5. Open field test

Locomotor activity was measured in the apparatus (100 cm × 100 cm × 50 cm), made of white wood (all sides), divided by red lines into 25 squares of 20 cm × 20 cm positioned in a quiet room. The test room was illuminated at the same intensity as the colony room.

Each mouse was placed in the center of the open field, and its behavior was observed for 10 min. Total locomotion (the total number of squares crossed), peripheral locomotion (the number of outer squares, those adjacent to the walls, crossed), and central locomotion (the number of inner squares crossed), were measured.[17] Quinine at doses of 20, 30, 40, 50, 60 mg/kg, diazepam at dose of 3 mg/kg, or normal saline + Tween 80 (10 ml/kg) injected i.p. into mice of each group, 30 min before starting the experiments, respectively.

2.5.1. Statistical analysis

The data were expressed as mean values ± S.E.M. and tested with analysis of variance (ANOVA) followed by the multiple comparison test of Tukey–Kramer. Results with $P < 0.05$ were considered significant.

3. Results

In PTZ model in this current study, quinine at the dose of 60 mg/kg significantly increased the latency of seizure compared to control ($P < 0.05$). However, quinine at the doses of 40, 50, 60 mg/kg decreased the duration of seizure in a dose-dependent manner ($P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively) (Table 1). Diazepam (0.5–1 mg/kg), the positive control, significantly increased the latency of seizure and decreased the duration of seizure compared to control ($P < 0.001$, and $P < 0.001$, respectively).

In the potentiation of sodium pentobarbitone sleep test, quinine significantly increased the sleeping time in mice at doses of 50 and 60 mg/kg compared to normal saline + Tween 80 control in a dose-dependent manner ($P < 0.01$, and $P < 0.001$, respectively) (Table 2). Also, quinine at the doses of 50 and 60 mg/kg significantly decreased the latency to sleep compared to control in a dose-dependent manner ($P < 0.05$, and $P < 0.01$, respectively) (Table 2). Furthermore, diazepam at 1 mg/kg significantly increased the sleeping time in mice and decreased the latency compared to control ($P < 0.001$).

Table 1
Anticonvulsant effect of quinine in the pentylenetetrazole-induced convulsion in mice

Treatment (dose)	Onset (s)	Duration (s)	Seizure protection (%)	Mortality protection (%)
Control	51.83 ± 1.64	12 ± 1.80	0	0
Diazepam (0.5 mg/kg)	485.5 ± 74.97***	3.5 ± 2.21***	80	90
Diazepam (1 mg/kg)	600 ± 0***	0 ± 0***	100	100
Quinine (20 mg/kg)	47 ± 2.5	8.8 ± 1	0	0
Quinine (30 mg/kg)	49.3 ± 3.9	10.7 ± 1	0	0
Quinine (40 mg/kg)	49.5 ± 4.5	4 ± 1.5**	0	0
Quinine (50 mg/kg)	169.2 ± 71.9	5 ± 1**	20	20
Quinine (60 mg/kg)	399.4 ± 98*	0 ± 0***	50	30

Control (normal saline + Tween 80); control, diazepam and quinine were administered i.p. 30 min before the injection of PTZ (90 mg/kg, i.p.); values are the mean ± S.E.M. for 10 mice.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, compared to control group, Tukey–Kramer test.

Table 2
Potentiation of the pentobarbital sleep with quinine in mice

Treatment (dose)	Latency (min)	Duration (min)
Control	8.3 ± 1.9	20.3 ± 1.6
Diazepam (1 mg/kg)	2.4 ± 0.2***	95.71 ± 6.3**
Quinine (20 mg/kg)	9.4 ± 1.1	30.7 ± 9.7
Quinine (30 mg/kg)	5.9 ± 0.5	39.6 ± 9.1
Quinine (40 mg/kg)	5.8 ± 0.6	42.8 ± 4.6
Quinine (50 mg/kg)	4.9 ± 0.3*	88.4 ± 12.9**
Quinine (60 mg/kg)	3.7 ± 0.4**	173.27 ± 34.4***

Control (normal saline + Tween 80); control, diazepam and quinine were administered i.p. 30 min, before pentobarbital (30 mg/kg). Mean latency and duration of sleep in min ± S.E.M. from 10 mice in each group.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared to control, Tukey–Kramer test.

Quinine 40–60 mg/kg reduced total locomotion, as well as peripheral and central locomotion compared to the control group (all $Ps < 0.001$) (Fig. 1). Diazepam (3 mg/kg) significantly decreased total locomotion, as well as peripheral and central locomotion compared to the control group ($P < 0.001$) (Fig. 1).

4. Discussion

Our results indicate that quinine has anticonvulsant activities in PTZ model. The inhibitory effect of quinine on the duration of seizure appeared at lower doses than 60 mg/kg. However, it appears that quinine can specifically inhibit both induction and duration of seizure at a dose of 60 mg/kg. Thus, it seems that quinine, by blocking gap junction channels, inhibits both the induction and duration of epilepsy in the PTZ model of seizure. Similarly, our data showed that quinine, similar to another gap junction blocker, carbenoxolone, has anticonvulsant effects in PTZ model.[14] In line with this view, recently Bostanci and Bagirici have shown that quinine may act as an antiepileptic drug in animal models of epilepsy *in vivo*. It significantly decreased spike frequencies, spike amplitudes, and epileptic behavioral score in penicillin-induced generalized epileptiform activity. In this *in vivo* epilepsy study quinine did not alter baseline EEG activity.[12] It has been suggested that quinine may decrease epileptiform activity via preventing gap junction mediated communication between neurons and furthermore, that the use of specific gap junction blockers might be useful in the treatment of epilepsy by reducing or even preventing the propagation and synchronization of epileptiform activity.[12]

In a previous study, our results demonstrated that carbenoxolone, at high doses, causes anticonvulsant effects that may be related to unspecific actions on gap junction channels. Quinine, in another previous study, was shown to close gap junction channels in a reversible, concentration-dependent and connexin-specific manner at an intracellular binding site.[7] These results, taken together, support the proposed role of gap junction channels in the generation of seizures and are the first reported results to demonstrate the efficacy of gap junction blockade in a model of generalized clonic, or tonic clonic, seizures *in vivo*. Seizures have traditionally been recognized as a symptom of abnormal neuronal synchronization, and until recently have been thought to be a result of aberrant synaptic communication.[4] Modeling studies indicate that neuronal synchronization can be mediated by low densities of gap junctions either between dendrites, as long as these dendrites are excitable, or between the axons of pyramidal cells.[18,19] Furthermore, the electrotonic coupling could be promoted during epileptogenesis.[20,21] It has been shown that targeted focal delivery of gap junction blockers significantly reduces percentage of seizure time in a model of epilepsy that is resistant to traditional treatment, including treatment with high-dose phenytoin, diazepam, and focally delivered tiagabine and does so without significant systemic side effects.[4] Gigout and colleagues have shown that gap junction channels play a role in synchronizing human neocortical networks and, similar to other studies, have shown that these channels may initiate epileptiform activity in focal cortical dysplasia (FCD).[22] It has been reported that GABA reduces gap junction-mediated communications between suprachiasmatic neurons by interacting with $GABA_A$ receptors, allowing an increase in the influx of Cl^- thus, altering electrical properties of the cell membranes.[23] Moreover, there is strong evidence for the role of the GABAergic system in modulating gap junction channels: Muscimol, a $GABA_A$ receptor agonist, demonstrated uncoupling effects in a dose-dependent manner and these effects were abolished by application of bicuculline.[18,24] However, there are several controversial reports about the role of GABA receptor-mediated mechanisms in initiating and maintaining epileptiform synchronization.[25,26] Cx36 is expressed in GABAergic interneurons in several brain regions.[8] Spontaneous field inhibitory post-synaptic potentials (IPSPs) and GABAergic ictal-like events were completely and reversibly blocked by quinine.[26] In this context, another gap junction blocker, carbenoxolone, also suppressed inhibitory IPSPs generated by rodent CA3 pyramidal cells in presence of the K^+ channel blocker 4-aminopyridine (4AP).[27] Thus, although it is possible that quinine expresses its anticonvulsive effects via preventing gap junction mediated communication between neurons in generalized clonic or tonic clonic seizures; it is also possible that quinine exerts its antiseizure effect via another independent mechanism.

In this current study at anticonvulsant doses, quinine produced a hypnotic effect in the pentobarbitone sleep test. This effect was similar to the hypnotic effect of carbenoxolone in a previous study.[14] Also, the effects of quinine on the locomotor activity were evaluated by open field test; a test used extensively for examining

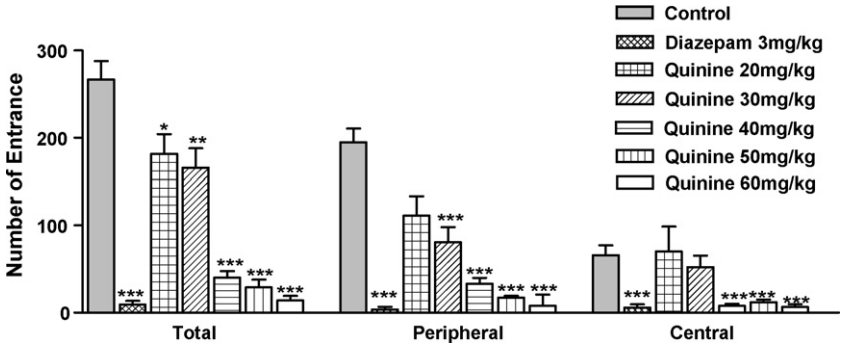

Fig. 1. Effects of quinine on open field test. Control (normal saline + Tween 80), quinine and diazepam (i.p.) were injected 30 min, before open field test. Data were reported as mean ± S.E.M., $n = 10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Tukey–Kramer test.

the behavioral effects of drugs and anxiety.[28] As a result, it appeared that quinine significantly reduced motor activity in mice at the doses required to suppress seizures. A similar result has been observed with carbenoxolone.[14]

Synchronized firing is a widespread phenomenon in the mammalian brain,[29,30] including the motor cortex,[31] respiratory motor neurons,[32,33] and limb motor neurons.[34,35] Collectively, these studies have demonstrated the presence of gap junctions at many levels of the motor system, in both motor neurons and in premotor pattern generating circuits. Further, gap junction coupling has been shown to bring about robust coordination patterns, even in the absence of chemical synapses, and has been shown to mediate synchronization of neurons during motor behaviors.[36] One possibility exists that the effects of quinine on seizure characteristics might be secondary to the effects of quinine on behavior or on sleep. At least, clear behavioral effects and "sleep" promoting effects have been shown here.

Moreover, quinine is widely used as an effective therapy for idiopathic leg cramps. But, the mechanism for this effect is unknown. It appears to decrease the excitability of the motor end plate, thereby reducing muscle contractility.[37] Therefore, it is possible that quinine by blocking these channels has significant effects on locomotor performance. However, a demonstration of whether these channels are, in fact, capable of mediating electrical transmission requires physiological experimentation.

5. Conclusions

In brief, the present study provides evidence for anticonvulsant activity of quinine in the generalized clonic seizure of PTZ model. At anticonvulsant doses, it also demonstrated hypnotic effects and decreased locomotion. As a result of these finding, we suggest that gap junctions represent an appropriate target for the development of drugs aimed at decreasing epileptiform synchronization and preventing epileptogenesis. It is suggested that structure–activity studies of quinine will perhaps lead to the synthesis of a quinine-based derivative that will be effective in treatment of seizure disorders in humans.

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