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Antiepileptic Properties of Alpha-asarone from Acori Graminei Rhizoma in Mice and Rats Seizure Models

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Abstract: α -asarone is a major essential oil component of rhizomes of *Acori Graminei Rhizoma* (AGR), a traditional medicinal plant utilized in China to treat epilepsy. The objective of present study was to investigate the antiepileptic activity of α -asarone in various animal seizure models. Experimental seizure models were established in mice and rats in which the antiepileptic properties of α -asarone were compared with those of Valproic Acid (VPA), Carbamazepine (CBZ) and Clonazepam (CNP). The Maximal Electroshock Seizure (MES) test and Subcutaneous Pentylentetrazol Seizure (scPTZ) test were done in Kunming (KM) mice. The lithium-pilocarpine model was employed to assess the antiepileptic activity in rats. The seizure incidence significantly decreased by 40-100% in the MES test, 50-90% in the scPTZ test and 40-80% in the lithium-pilocarpine model; the seizure latency dramatically prolonged by 180 sec in the scPTZ test and 4-15 min in the lithium-pilocarpine model; the seizure severity score was markedly reduced by 1.96 in the lithium-pilocarpine model. The seizure frequency markedly reduced in the lithium pilocarpine model. In addition, significant differences in the above variables were noted between α -asarone at 200 mg kg⁻¹ (p.o.) and that at 50 or 100 mg kg⁻¹. These results suggest that α -asarone has favorable antiepileptic activity, is an active antiepileptic drug and has potential implication in the management of epilepsy.

Key words: Antiepileptic drug, α -asarone, maximal electroshock seizure, subcutaneous pentylentetrazol seizure, lithium, pilocarpine, epilepsy, animal model

INTRODUCTION

Epilepsy, a common neurological disorder, is characterized by abnormal discharge of cerebral neurons and manifested as various types of seizures (Chang and Lowenstein, 2003). Different types of seizures have distinct neurobiological conditions and can be treated with different medications (Arts and Geerts, 2009). Although the judicious use of presently available Antiepileptic Drugs (AEDs) allows 70-80% of epileptic patients to be seizure-free, nearly 20-30% of patients who develop partial and secondary generalized seizures are still refractory to treatment with current AEDs (Granata *et al.*, 2009; Kwan *et al.*, 2011). In addition, severe adverse effects are common with most of AEDs (Dalkara and Karakuri, 2012). As a consequence, it is imperative to develop new AEDs with high efficacy but less adverse effects.

Recently, the alternative management of epilepsy in terms of Chinese medicine becomes attractive to patients as well as physicians in both China and Western countries. In traditional Chinese medicine, the roots and rhizomes of *Acori Graminei Rhizoma* (AGR) has been recommended for centuries for the treatment of epilepsy, or in combination with other medical herbs for the improvement of learning and memory (Zhang *et al.*, 2007). Modern pharmacological studies have also shown that some extracts of AGR may induce sedation, decrease spontaneous activity, potentiate pentobarbital-induced sleeping time and possess antiepileptic and spasmolytic activities (Liao *et al.*, 1998). However, the major components possessing the antiepileptic properties are still poorly understood.

α -asarone [trans-1-propenyl-2,4,5-trimethoxybenzene] is found in diverse vegetals including Araceae plants (*Acorus*) and Annonaceae tree (*Guatteria guameri*)

Greenman) (Pages *et al.*, 2010). Vegetal extracts containing this active component have been used in Indian, Korean and Mexican traditional medicines due to their antiepileptic and neurological properties as well as diuretic, hypocholesterolemic, anticholelithiasis and anxiolytic effects (Vohora *et al.*, 1990; Chen, 1984; Shukla *et al.*, 2002; Garduno *et al.*, 1997; Liu *et al.*, 2012).

In the present study, we investigated the antiepileptic profile of α -asarone of AGR in experimental mouse and rat seizure models, in which the traditional Maximal Electroshock Seizure (MES) test, subcutaneous pentylenetetrazol seizure (scPTZ) test and lithium-pilocarpine model of chronic epilepsy were employed and the antiepileptic activities of α -asarone were compared with those of traditional AEDs including VPA, CBZ and CNP.

MATERIALS AND METHODS

Animals: Male Kunming (KM) mice weighting 20-30 g and male Wistar rats weighting 200-250 g, were used in the present study. The animals were given *ad libitum* to standard laboratory diet and tap water and maintained in an environment with 12/12 h light/dark cycle, ambient temperature of $23\pm 2^\circ\text{C}$ and humidity of $55\pm 20\%$. Animals were allowed to acclimate to the environment for ≥ 5 days. The whole protocol was approved by the Committee of Experimental Animal Administration of our University and experiments were performed in accordance with the National Institutes of Guide for Care and Use of Laboratory Animals.

Chemicals: α -asarone (Shengyang Aisheng Pharmaceutical Co Ltd., China) and diazepam (Hubei Pharmaceutical Factory, China) were used in the present study. Pentylenetetrazol (PTZ; Sigma, USA), lithium chloride (Sigma, USA) and pilocarpine nitrate (Sigma, USA) were used as the convulsants in this study and dissolved in normal saline. VPA, CBZ and CNP were used as control AEDs, suspended in normal saline and orally administered at 10 mL kg^{-1} . In the control group, animals were treated orally with normal saline of equal volume.

Assessment of antiepileptic activity

MES test in mice: MES (sine wave, 2 Hz, 100 V, 0.25 sec) was introduced via electric stimulation with ear electrodes connected to an electric stimulator (YSD-4G) and the tonic hindlimb extension was qualified as MES. One hundred MES mice were subdivided into 5 groups ($n = 20$ per group) (control group, 50 mg kg^{-1} α -asarone group, 100 mg kg^{-1} α -asarone group, 200 mg kg^{-1} α -asarone

group and VPA group). In the α -asarone groups, α -asarone was orally administered to mice at $50\text{-}200\text{ mg kg}^{-1}\text{ day}^{-1}$. In the control group, animals orally received normal saline of equal volume while those in the VPA group underwent treatment with oral VPA at $200\text{ mg kg}^{-1}\text{ day}^{-1}$. Treatment was performed twice daily for 28 days. Seizures were induced at 60 min after the last treatment and the animals exhibiting tonic hindlimb extension was recorded. Animals failing to show tonic hindlimb extension were regarded as protected ones (Kitano *et al.*, 2005).

scPTZ test in mice: PTZ was administered subcutaneously at 85 mg kg^{-1} . The animals were placed back to cages and observed for 60 min for seizures. The presence of clonic convulsion indicated successful establishment of scPTZ model. One hundred scPTZ mice were subdivided into 5 groups ($n = 20$ per group) (control group, 50 mg kg^{-1} α -asarone group, 100 mg kg^{-1} α -asarone group, 200 mg kg^{-1} α -asarone group and CNP group). The treatment in 3 α -asarone groups and control group was similar to those abovementioned. In the CNP group, CNP was orally administered at $2\text{ mg kg}^{-1}\text{ day}^{-1}$. Treatment was performed twice daily for 28 days. Seizures were induced at 60 min after the last treatment and the animals exhibiting clonic convulsion and the latency to the first clonic seizure were recorded. Animals failing to show clonic seizure were regarded as protected ones (Obay *et al.*, 2007).

Lithium-pilocarpine model of chronic epilepsy in rats:

Adult Wistar rats were intraperitoneally injected with pilocarpine to induce Status Epilepticus (SE), a model of injury-induced epilepsy. Lithium chloride was injected at 3 meq kg^{-1} (125 mg kg^{-1}) intraperitoneally at 18-24 h before pilocarpine treatment. Furthermore, rats were first injected with methylscopolamine-bromide ($1\text{ mg kg}^{-1}\text{ i.p.}$), to prevent the peripheral effects of pilocarpine. The pilocarpine ($40\text{ mg kg}^{-1}\text{ i.p.}$) was then administered at 30 min after methylscopolamine treatment (Muller *et al.*, 2009). Approximately 30 min after pilocarpine treatment, rats began to manifest motor seizures. Seizures were scored according to a modified Racine scale and only motor seizures were scored (Medina-Franco *et al.*, 2005). Seizures were scored as follows: class III, rats displayed forelimb clonus with a lordotic posture; class IV, rats reared with a concomitant forelimb clonus; class V, rats had characteristics in class IV and fell down. After pilocarpine injection, animal were placed back to cages and observed in isolation for at least 3 h. All motor seizures as well as behaviors were recorded. The

incidence of seizures and latency to the first epileptic seizure, motor SE and generalized tonic-clonic seizures were recorded. One hour after continuous motoring SE, diazepam was administered (10 mg kg⁻¹, i.p.) to terminate the seizures. Once the seizures stopped, all rats were injected subcutaneously with about 3 mL of lactated Ringer's solution after the pilocarpine-induced SE. The rats were housed in an environment with 12 h/12 h light/dark cycle and given *ad libitum* access to food and water for 2 months in the presence of direct monitoring of spontaneous seizures. The latency to the occurrence of Spontaneous, Recurrent Seizures (SRS) was daily video-recorded for 8 h at daytime for 4 weeks before these rats were used in the investigations on the effects of α -asarone. The Racine scale was also used to score the spontaneous seizures as abovementioned. Rats with SRS were selected for further experiments.

One hundred rats with SRS were subdivided into 5 groups (n = 20 per group) (control group, 50 mg kg⁻¹ α -asarone group, 100 mg kg⁻¹ α -asarone group, 200 mg kg⁻¹ α -asarone group and CBZ group). Treatment in 3 α -asarone groups and the control group was similar to those abovementioned. Animals in the CBZ were treated with CBZ at 200 mg/kg/day. Treatment was done twice daily for 28 days. The SRS frequency was daily video-recorded for 8 h at daytime for 4 weeks. Seizures were induced at 60 min after the last administration. The latency to the first seizure, the number of animals exhibiting seizures of class III-V and the seizure scores were recorded. Animals failing to show seizures of class III-V were regarded as protected ones.

Statistical analysis: Data in each experiment were normalized by those at baseline. All data were presented as Mean±standard error (SEM). Comparisons among different groups were done with analysis of variance (ANOVA) followed by Dunn's test (two-sided). All analyses were performed using the SPSS version 11.0 for Windows. A value of p<0.05 was considered statistically significant.

RESULTS

Effects of α -asarone on MES: As shown in Table 1, α -asarone significantly decreased the incidence of tonic hindlimb extension induced by electroshock. All animals receiving vehicle after electrical stimulation (n = 20), 40% of animals receiving 50 mg kg⁻¹ α -asarone (n = 8) and 20% of animals receiving 100 mg kg⁻¹ α -asarone (n = 4) presented with seizures, but all animals were free of seizures in the 200 mg kg⁻¹ α -asarone group. The seizure incidence and mortality were markedly reduced after treatment with α -asarone at different concentrations.

Table 1: Effect of α -asarone on MES in mice

Groups (mg kg ⁻¹)	Seizure rate (%)	Mortality (%)
α -asarone: 50	40 ^a	30 ^a
α -asarone: 100	20 ^b	10 ^b
α -asarone: 200	A ^b	A ^b
VPA: 200	A ^b	A ^b
Control	100	40

^ap<0.05, ^bp<0.001 vs. control group; A: Absence of convulsions, n = 20

Table 2: Effect of α -asarone on PTZ induced seizure in mice

Groups (mg kg ⁻¹)	Seizure parameters	
	Incidence (%)	Latency (sec)
α -asarone: 50	50 ^a	348±57 ^a
α -asarone: 100	30 ^b	402±69 ^b
α -asarone: 200	10 ^b	422±78 ^b
CNP: 2	A ^b	A ^b
Control	100	237±44

^ap<0.05, ^bp<0.001 vs. control group; A: Absence of convulsions, n = 20

Table 3: Effect of α -asarone on lithium-pilocarpine induced seizures in rats

Groups	Seizure parameters			
	Incidence (%)	Latency (min)	Score	Seizure frequency
α -asarone: 50	60%	25.37±4.60 ^a	3.38±1.63 ^a	14.12±4.98
α -asarone: 100	40%	31.20±5.42 ^b	2.44±1.16 ^b	13.24±4.56
α -asarone: 200	20%	36.42±8.14 ^b	2.16±0.74 ^b	10.28±4.36 ^c
C ^z : 200	A	A ^b	A ^b	9.45±3.22 ^a
Control	100%	21.50±6.69	4.12±0.35	14.26±5.16

^ap<0.05, ^bp<0.001 vs. control group; A: Absence of convulsions, n = 20

Effects of α -asarone on PTZ-induced seizures: All animals (n = 20) treated with vehicle presented with generalized seizures (Table 2). The latency to the first seizure was 237±44 sec. In addition, 50% of animals receiving 50 mg kg⁻¹ α -asarone (n = 10), 30% of animals receiving 100 mg kg⁻¹ α -asarone (n = 6) and 10% of animals receiving 200 mg kg⁻¹ α -asarone (n = 2) presented with seizures. The latency to the first seizure was significantly increased in 3 α -asarone groups (348±57 sec for 50 mg kg⁻¹, 402±69 sec for 100 mg kg⁻¹ and 422±78 sec for 200 mg kg⁻¹).

Effects of α -asarone on lithium-pilocarpine induced seizures: All rats developed SRS with the latency of 15.8±8.8 days. Administration of 40 mg kg⁻¹ pilocarpine (n = 20) at 18-24 h after injection of 125 mg kg⁻¹ lithium chloride induced seizures in each animal in the control group, with the mean latency to the first seizure of 21.50±6.69 min (Table 3). The mean seizure score was 4.12±0.35 (n = 20). Only 60% of animals receiving 50 mg kg⁻¹ α -asarone (mean seizure score, 3.38±1.63; latency to seizure, 25.37±4.60 min, n = 12), 40% of animals receiving 100 mg kg⁻¹ α -asarone (mean seizure score, 2.44±1.16; latency to seizure, 31.20±5.42 min, n = 8) and 20% of animals receiving 200 mg kg⁻¹ α -asarone (mean seizure score, 2.16±0.74; latency to seizure, 36.42±8.14 min, n = 4), had presented with seizures. The seizure incidence,

latency and score were significantly reduced after treatment with α -asarone at different concentrations. Moreover, the seizure frequency was significantly reduced in the 200 mg kg⁻¹ α -asarone group, but no significant difference was observed between 50 or 100 mg kg⁻¹ α -asarone group and control group.

DISCUSSION

α -asarone is a major essential oil component of rhizomes of AGR, a traditional medicinal plant utilized in China to treat lunatic, narcosis and chronic tracheitis. α -asarone has attracted widespread interest and is recently used as a potential antithrombotic, antimicrobial, insecticidal, nematocidal and antifeedant agent (Medina-Franco *et al.*, 2005). Animal studies have demonstrated various pharmacological characteristics of α -asarone in the nervous system, such as anticonvulsive, sedative and hypothermic effects (Liao *et al.*, 2005). Intragastric administration of α -asarone to young rats can suppress PTZ induced seizures and inhibit the abnormal discharge of frontal cortex by decreasing the expression of glutamic acid receptor (NMDAR1) in the hippocampal neurons (Cho *et al.*, 2001).

In previous study, Liao *et al.* (2005) has demonstrated that a single administration of essential oil (1.25 g kg⁻¹), of AGR decreased the convulsive rate significantly in the MES model. It was failed to prevent seizures in the PTZ seizure model (Liao *et al.*, 2005). However, in that study, α -asarone was acutely administered. The present study focused on the antiepileptic activities of chronic treatment with α -asarone and its implication in the clinical antiepileptic therapy. Our results demonstrated that α -asarone exerted good antiepileptic effect in the MES test, scPTZ test and lithium-pilocarpine induced seizures. The antiepileptic spectrum of α -asarone was similar to that of VPA, CNP and CBZ. At the effective concentrations, the antiepileptic effect of α -asarone was lesser potent than that of VPA, CNP and CBZ. Though asarone shows effective antiepileptic properties, the effects are of lesser magnitude than conventional AEDs.

α -asarone was also reported to increase the γ -aminobutyric acid (GABA) level but decrease the glutamate level in the brain of seizure animals (Koo *et al.*, 2003). The antiepileptic mechanisms of α -asarone are largely unknown. Studies have shown that some drugs can increase the cerebral GABA exhibiting antiepileptic activity against seizures induced by MES, PTZ and lithium-pilocarpine (Meldrum *et al.*, 2007). Enhancement of GABAergic systems has been found to be involved in the pharmacological action of several AEDs, particularly VPA, Benzodiazepines (BZD) and barbiturates

(Mula, 2011; Sankar and Holmes, 2004). In addition, Pages *et al.* (2010) proposed that the activities of α -asarone in various animal seizure models were related to its antioxidant properties (Pages *et al.*, 2010) and studies have demonstrated that extracts containing α -asarone have anti-radical activity towards 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and, on the other hand, with inhibitory properties towards lipid peroxidation (Koo and Lee, 2001; Koo *et al.*, 2003). The exact mechanism by which the α -asarone exhibits the antiepileptic effect remains to be determined.

There is evidence showing that epilepsy patients usually have cognitive impairment, especially memory disturbance, due to the epilepsy itself and/or the adverse effects of AEDs in the long-term antiepileptic therapy (Motamedi and Meador, 2003). Cognitive disturbance is one of the major factors influencing the quality of life in epilepsy patients (Brunbech and Sabers, 2002; Kerr *et al.*, 2011). The AEDs with cognition-enhancing activity or α -asarone in combination with AEDs might be beneficial for the antiepileptic therapy. The α -asarone has been reported to improve the cognition and exert protective effect on seizures in epilepsy patients (Cho *et al.*, 2000; Lee *et al.*, 2003). Based on these findings in the pharmacological profile of α -asarone, we speculate that α -asarone is beneficial for epilepsy patients.

CONCLUSION

In summary, α -asarone possesses antiepileptic activity against the MES, PTZ and lithium-pilocarpine induced seizures. The antiepileptic spectrum of α -asarone was similar to that of VPA, CNP and CBZ. Our findings suggest that α -asarone is an orally active AED and has potential implications in the antiepileptic therapy. Further researches are required to confirm the exact mechanism underlying the antiepileptic effect of α -asarone.

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