Invest Clin 48(3): 349 - 358, 2007

On the anticonvulsant activity of kaurenic acid.

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Key words: Diterpenes, kaurenic acid, seizure, anticonvulsant, sudden cooling.

Abstract. Kaurenic acid [(-)-kaur-16-en-19-oic acid] is a diterpene isolated from the aerial parts of *Espeletia semiglobulata*, one of 85 species of Espeletiinae found in Venezuela. Its anticonvulsive activity was studied using two different models of experimental seizures: spinal seizures induced by sudden cooling (SSSC) in amphibians and seizures induced by pentylenetetrazol (PTZ) in mice. In SSSC, kaurenic acid (KA) inhibited the tonic hind-limb extension with an ED50 of 2.5 mg/kg. It was 4-fold more potent than known anticonvulsant drugs such as carbamazepine and phenytoin and 100-fold more potent than valproic acid. However, KA as well as valproic acid were ineffective against the clonic phase of SSSC. In the PTZ-induced seizures, KA at doses of 0.625 and 1.25 mg/kg increased the latency of seizure onset and protected against generalized clonic-tonic seizures by 45% and 65%, respectively. The sedative effects of KA had an ED50 of 8.5 mg/kg in mice and 75 mg/kg in amphibians. This work provides experimental evidence supporting the potential value of kaurenic acid as an anticonvulsive drug.

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Sobre la actividad anticonvulsiva del ácido kaurénico.
Invest Clin 2007; 48(3): 349 - 358

Palabras clave: Diterpenos, ácido kaurénico, convulsión, anticonvulsivo, enfriamiento rápido.

Resumen. El ácido kaurénico [(-)-kaur-16-en-19-oic acid] es un diterpeno aislado de las partes aéreas de la planta *Espeletia semiglobulata*, una de la 85 especies de Espeletiinae encontradas en Venezuela. El efecto anticonvulsivo del ácido kaurénico fue estudiado empleando dos modelos diferentes de convulsiones experimentales: convulsiones espinales inducidas por enfriamiento brusco (SSSC) en anfibios y convulsiones inducidas por pentilenotetrazol (PTZ) en ratones. En SSSC, el ácido kaurénico (KA) inhibió la fase tónica con una ED50 de 2,5 mg/kg. KA fue cuatro veces más potente que anticonvulsivos conocidos tales como carbamazepina y fenitoína y 100 veces más potente que el ácido valproico. Sin embargo, el KA al igual que el ácido valproico, fueron inefectivos contra la fase clónica de las SSSC. En convulsiones inducidas por PTZ en ratones, el KA aumentó la latencia y disminuyó la incidencia de la fase clónica-tónica generalizada de las convulsiones inducidas por PTZ en 45% y 65%, a dosis de 0,62 y 1,25 mg/kg, respectivamente. KA produjo sedación a una dosis efectiva (ED50) de 8,5 mg/kg en los ratones y de 75 mg/kg en anfibios. Este trabajo aporta evidencia experimental que soporta el valor potencial del KA como una droga anticonvulsiva.

Received: 17-05-2006. Accepted: 30-11-2006.

INTRODUCTION

An intense search for new antiepileptic agents has been the focus of many investigators in the last three decades, aiming to treat some types of generalized clonic-tonic seizures that are resistant to drug therapy. In this concern, some attention has been gained by diterpenes, a group of natural products of the terpene class containing 20 carbon atoms and 4 branched methyl groups. This occurred specially after the report that forskolin prevents pentylenetetrazol (PTZ)-induced seizures (1). This work was published after the discovery that forskolin, isolated from the root of *Coleus forskohlii*, is a potent diterpene activator of adenylate cyclase. Forskolin increases the intracellular level of cAMP and produces subsequent activation of cAMP-dependent protein kinases involved in the biological responses to many receptor agonists (2). However, some studies about the anticonvulsant activity of diterpenes are contradictory (3-5).

Kaurenic acid [(-)-kaur-16-en-19-oic acid], is a diterpene isolated from the aerial parts of *Espeletia semiglobulata* (6). We have previously found that *E semiglobulata* is the species from which kaurenic acid (KA) is easily isolated because its resin is rich in this acid and it contains very little grandiflorenic acid, a compound which makes purification difficult (7).

KA from different natural sources is under investigation for possible antibacterial (8), cytotoxicity (9) and anti-inflammatory activities (10). In the course of several

experiments designed to investigate the anti-inflammatory effect of KA using rats and mice, we noticed that treated animals exhibited some degree of sedation and somnolence. Since these are common side effects produced by most antiepileptic drugs, we decided to screen a possible anticonvulsant activity of KA by using two animal models: a) In amphibians, the spinal seizure-induced by sudden cooling (SSSC), attributed to release of excitatory amino acids, glutamate, aspartate and the co-agonist glycine (11, 12), in which common anticonvulsant drugs are active at a similar dose range that maximal electroshock seizure model (13); b) in mice, the generalized seizure induced by the PTZ, a $GABA_A$ receptor antagonist, used even at high doses, in the screening of putative drugs with anti-absence activity (14).

MATERIALS AND METHODS

Isolation of kaurenic acid (KA)

Aerial parts of *E. semiglobulata*, 30 Kg, was collected at Páramo of Piedras Blancas, Mérida, Venezuela. The leaves were air dried, grounded and extracted several times with n-hexane at room temperature. The hexane extract was concentrated and shaken with a 0.5 N NaOH solution. The aqueous layer, which contained the sodium salt of KA as an emulsion was filtered in a Büchner funnel. The solid precipitate was mixed with water, acidified with diluted HCl and it was shaken with hexane. The KA recovered from the hexane layer was further purified by flash chromatography over silica gel using hexane and hexane/diethyl ether (9:1) as solvent. An aliquot of each chromatographic fraction was methylated and inspected by gas chromatography at 250°C (6).

Induction of seizures in amphibians

Experiments were performed using the isolated spinal cord-hind limb preparation of South American tropical toads (*Bufo marinus*) following the technique previously described (11). Amphibians were captured in the surrounding areas of the city of Barquisimeto. They were kept in open spaces for 1-2 weeks before used. After pithing, the spinal cord was separated from the brain at C1 level and kept in its vertebral canal joined to the hind limbs by the sciatic nerves.

The seizure was induced by placing the isolated spinal cord into a cold Ringer's bath maintained at 7°C using a bath circulator (Haake, model FK). The intensity of the seizure was assessed by recording the contractions of the gastrocnemius muscle using a myograph type B connected to a physiograph (Narco Biosystems). The latency of the seizure onset was defined as the time elapsed between the immersion of the isolated cord into the cold Ringer's bath and the visualization of the first clonic muscle contractions. Animals with similar body weight were selected in order to compare the latencies for seizure onset. The duration of seizure was determined by measuring on the recording paper the time from the appearance of the first group of muscle contractions until all muscle activity ceased. In this model, the pattern of recorded muscle contractions, latencies and duration of seizures were compared with common anticonvulsants drugs (13).

Induction of seizures by pentylenetetrazol (PTZ) in mice

Groups of 40-60 NMRI mice weighing 25 to 35 g were obtained from the Animal Facility of the Universidad Centroccidental Lisandro Alvarado, Barquisimeto and were acclimatized for 1 day before used. All experiments were performed in the morning. A dose of PTZ at 85 mg/kg, i.p. reported to induced convulsions in 96-98% of the animals was used (15, 16), The latency of the first generalized clonic seizure, as well as,

the number of animal that exhibited generalized clonic-tonic seizure was noted.

Anticonvulsant effect endpoints

In amphibians, the evaluation of the anticonvulsant effect was done using two endpoints: a) abolition of tonic hind limb extension (THE) and b) total blockade of seizure activity, i.e. no visualization neither of tonic nor clonic muscle contractions (13), whereas in the i.p. PTZ model the endpoint was the first episode of continuous generalized clonic-tonic seizure of fore- and/or hind limbs with loss of the righting reflexes, i.e. animals fell onto their side (17).

Evaluation of adverse effects

A general behavioral profile was used to evaluate sedation. In mice, sedation was noted when exhibited somnolence (decrease in motor activity) and decreased the righting reflexes, i.e. when mice were placed in their back (U shape) and delayed more than 5 sec to regain the normal position on their four feet pad. Motor impairment was present when mice showed weakness of the hind limbs. Amphibians were considered sedated when exhibited a decreased in the righting reflexes, i.e. more time to recover their normal position after animals were placed on their back. Motor impairment was considered present when animals were unable to walk and jump normally.

Injection of drugs

KA was dissolved in distilled water at a concentration of 6 mg/mL and stored in the refrigerator for no more than 2 weeks. In amphibians, KA was injected into the ventral lymphatic sac (i.l.) and mice were injected i.p. In amphibian, KA was injected 1 to 17 h before the induction of seizure in order to estimate its peak effect. Subsequently, in both models KA, was given 4 h before the induction of seizure. Carbamazepine and phenytoin were purchased from Sigma (St. Louis, MO); valproic acid from commercial sources (Valpron®, Farma, Caracas, Venezuela) and PTZ from RBI (Natick, MA). Valproic acid was dissolved in 0.65% saline, while carbamazepine and phenytoin were dissolved in DMSO plus 0.65% saline. Common anticonvulsants were administered i.l. 1 h before the induction of SSSC. Control amphibians received the respective solvent and control mice were injected with PTZ dissolved in 0.85% saline.

Statistical analysis

Data for latencies and duration of seizures were analyzed using one way ANOVA followed by Dunnett's test compared with control values in the amphibian model and Student's "t" test in the PTZ model as suggested (17). A $p < 0.05$ was considered as significant.

RESULTS

Isolation of kaurenic acid

In total, 43 g of KA were obtained from *E. semiglobulata* (Fig. 1), its melting point was 175-178°C and the chemical structure is presented in Fig. 1. The sodium salt of KA was fairly soluble in water; but the solution often required to be shaken before it was taken into the syringe for injection.

The spinal seizure-induced by sudden cooling (SSSC)

In control amphibians the immersion of the isolated spinal cord into a cold Ringer's bath induced a typical seizure recorded as muscle contractions that began with a latency of 84 ± 6.7 sec. Initially, it was observed as very tiny muscle fibrillations or tremors which were visualized, but difficult to record. After this initial phase, a group of larger clonic muscle contractions

appeared that increased in intensity until a full tonic hind-limb extensions (THE) was reached. This THE phase lasted 4 to 6 seconds, and it was followed by a second group of irregular clonic muscle contractions until all activity ceased. The mean total duration of the SSSC was 12.5 ± 4.1 sec (n = 17) (Fig. 2).

KA was remarkably effective for abolishing the THE at a dose as low as 0.62 mg/kg (Fig. 2), but total blockade of the clonic phase was not achieved even when a

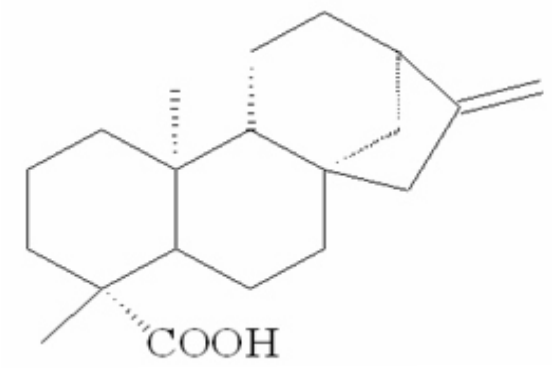

Fig. 1. Source and structure of kaurenic acid. This diterpene was isolated from the aerial parts of Espeletiinae, resinous plants that grow above 2500 meters of altitude in the Andes of Ecuador, Colombia, and Venezuela. About 85 species have been described for Venezuela and *Espeletia semiglobulata* (at the left) is the best source of kaurenic acid (at the right).

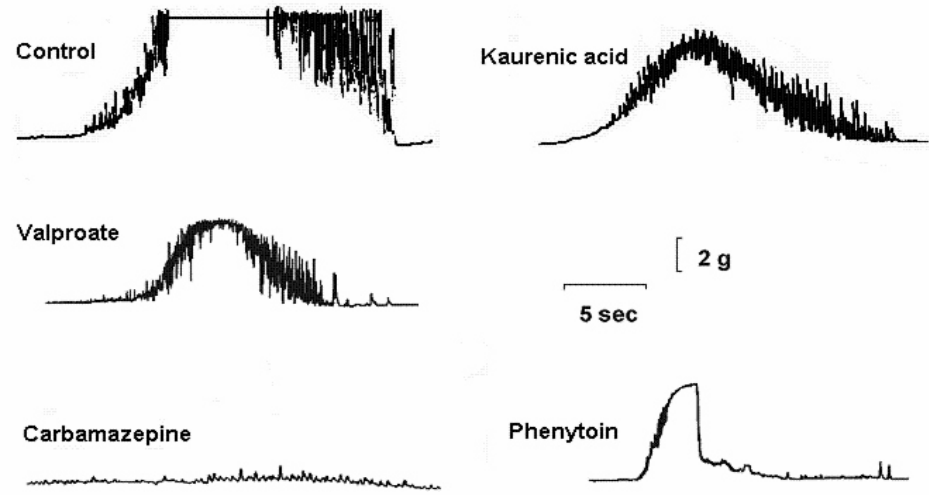

Fig. 2. Pattern of muscle contractions during SSSC. Control: Typical seizure in control toad. Note that during tonic hindlimbs extension (THE), the myograph exceeds its maximal capacity and a straight line is recorded. Recordings after kaurenic acid (2.5), valproic acid (500), carbamazepine (10) and phenytoin (5). Doses are expressed in mg/kg. THE was abolished after kaurenic acid and valproic acid and the clonic phase depressed after carbamazepine and phenytoin. Calibration of myograph and speed of recording shown in vertical and horizontal brackets applies to all recordings.

large dose of 160 mg/kg was given. The best peak effective time post-KA injection was observed to be at 4 h after injection; however, the blocking effect of KA could still be observed 17 h post injection (80 mg/kg, n = 4). When compared with common anticonvulsants, KA inhibited the THE with an ED50 of 2.5 mg/kg; while carbamazepine and phenytoin had an ED50 of 8.6 and 13.0 mg/kg, respectively (Fig. 5). Total blockade of seizure, (i.e. total depression of the clonic phase), was attained for carbamazepine and phenytoin with an ED50 of 12 and 16 mg/kg, respectively; while KA and valproic acid were ineffective even at doses of 160 and 1000 mg/kg, respectively.

KA did not alter the latency of seizure onset (i.e. the beginning of muscle contractions). Similar result was obtained when compared with valproic acid at 500 mg/kg; but carbamazepine and phenytoin significantly prolonged the latency at 5 and 10 mg/kg, respectively (Fig. 3).

KA had no activity against duration of the clonic phase of seizure and a similar effect was found when it was compared with valproic acid at doses of 50 and 100 mg/kg (Fig. 4); in contrast, carbamazepine and phenytoin tended to decrease the duration of the clonic phase at 5 mg/kg (Fig. 2).

Seizures induced by PTZ

In 14% of mice PTZ induced only clonic seizures, without loss of righting reflexes while generalized clonic-tonic seizure were observed in 86% of the animals (n = 22). When KA was previously given at doses of 0.62 (n = 18) and 1.25 (n = 20) mg/kg, KA protected mice against the generalized clonic-tonic seizure to 45% and 65% of the cases, respectively.

In animals treated with PTZ only the mean latency of seizure onset was 61 ± 9

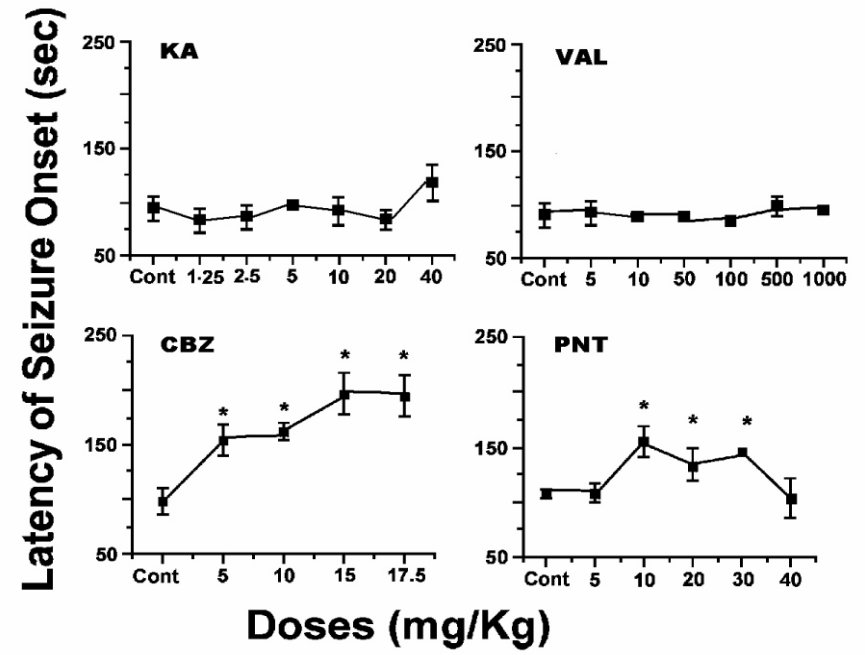

Fig. 3. Effect of kaurenic acid (KA) and major anticonvulsant drugs on latencies to onset of seizure. KA (overall ANOVA, F = 1.989, DF = 86, p = 0.058) and valproic acid (VAL) did not enhance the latency, whereas carbamazepine (CBZ) and phenytoin (PNT) increased the latency significantly. Each drug had its own control group and at least 6 toads were treated with each dose. Vertical bars indicated the SE of the mean. Asterisk is a $p < 0.05$.

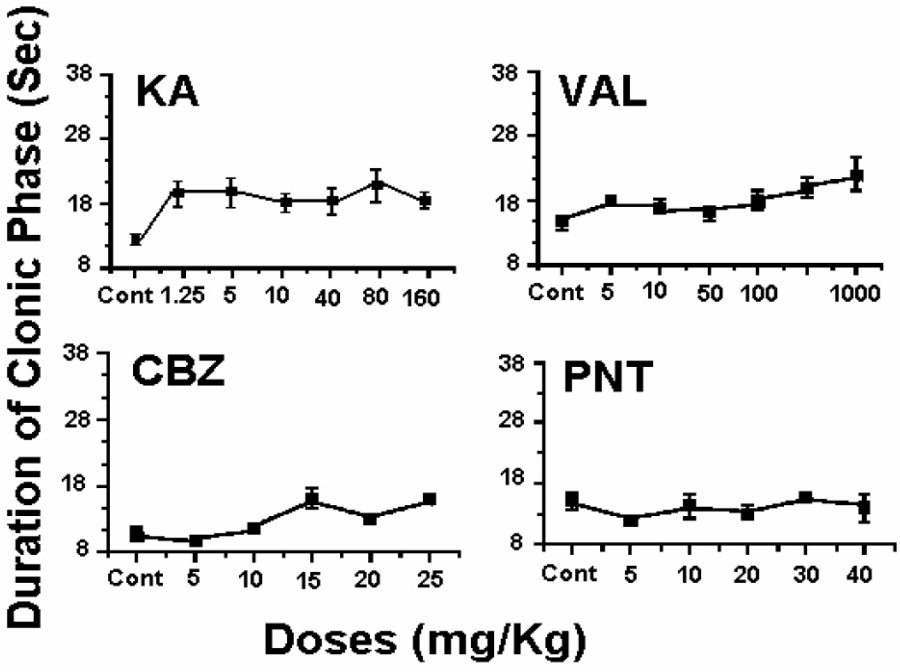

Fig. 4. Effect of kaurenic acid and major anticonvulsant drugs on duration of clonic phase of SSSC. Drug abbreviations are presented in previous figure. Although it is not significant, kaurenic acid (overall ANOVA, F = 1.109, DF = 80, p = 0.367) and valproic acid tend to increase the duration of the clonic phase. On the contrary, carbamazepine and phenytoin tend to decrease it. Each drug had its own control group.

sec. After pretreatment with KA at doses of 0.62 and 1.25 mg/kg respectively, the mean latency of seizure increased significantly to 291 ± 77 sec and 254 ± 55 sec ($p < 0.05$).

Estimation of adverse effects

In mice, KA at dose of 20 mg/kg, exhibited motor impairment that began 2 min after injection and long lasting signs of sedation (8 to 12 h) were seen in 70% of animals 30 min after treatment. While in amphibians, the sedative effect of KA was visible at 80 mg/kg, 30 min after treatment, but it lasted no more than 2 h (Fig. 5).

DISCUSSION

This work present evidences that KA, a diterpene isolated from *E. semiglobulata*, has a potent and remarkable activity against THE of SSSC in tropical toads and PTZ-induced clonic-tonic seizure in mice. After 4 h of KA administration the pattern of SSSC, recorded as muscle contractions, was very similar to that found after 1 h of valproic acid injection, i.e. both drugs abolished the tonic phase of the SSSCs, but failed to block the clonic phase or to alter the latency and duration of the clonic phase of SSSC. However, the action of KA was different from that of carbamazepine and phenytoin, wich were able to produce a total block of the clonic phase (13).

During SSSC, the THE is seen as a maximal muscle contraction, when into the spinal cord take place a large and long lasting depolarization accompanied of repetitive firing of motoneurons, that was recorded physiographically using the hemisected isolated spinal cord with a sucrose gap recordings (18). The repetitive firing is effectively abolished by N-methyl-D-aspartate (NMDA) receptor antagonists, but they only reduce

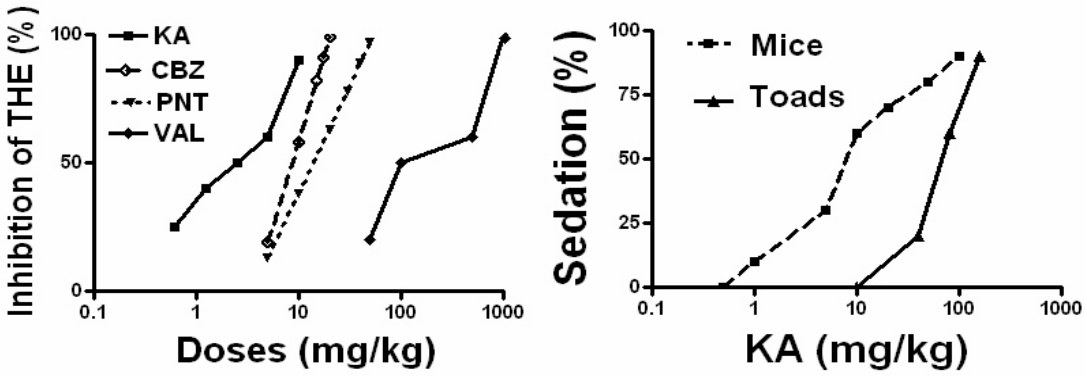

Fig. 5. Dose response curve comparison and adverse effect of kaurenic acid. The tonic hindlimb extension (THE) in the SSSC model was inhibited (ED50 in mg/kg) by: kaurenic acid 2.5, carbamazepine 8.6, phenytoin 13 and valproic acid 120. Sedative dose (ED50) in mice was 8.5 mg/kg and in toads 75 mg/kg. The inhibition of THE (%) or sedation (%) refer to the percentage of animals without exhibiting THE or showing sedation, within the respective group. Doses of drugs in X axis are in log scale. Drug abbreviations are indicated in Fig. 3.

the long lasting depolarization about one half (18). Furthermore, when NMDA receptor antagonists are injected and, their effect on SSSC recorded as muscle contraction, we have been able to see prolonged and weak clonic muscle contractions that last up to 28 sec (11, 13, 19) These previous findings let us think that KA in the SSSC model may not be acting as a NMDA receptor antagonist.

PTZ is the most commonly used chemical convulsant acting as $GABA_A$ receptor antagonist used to induce seizure in rodents. Several routes of administration and dosage regimes are used, but the most popular are: a) low PTZ doses (20-30 mg/kg) that induced absence-like seizure that requires EEG monitoring which is the major obstacle at these doses (14); b) intermediate subconvulsant doses of PTZ (40 – 60 mg) are used for investigation of proconvulsant action of drugs (3, 20) and c) high doses of PTZ (80 – 100 mg/kg, s.c.) which can induce generalized clonic seizure in all animals (15-17). Even though these high doses do not meet the criteria for experimental absence seizure, these clonic seizures are used to screen for anti-absence activity and are reported to produce recruitment of brainstem circuitry with resultant tonic seizures (14). After PTZ (85 mg/kg, s.c.) latencies to the onset of seizure was reported between 60–210 sec (15), but smaller latencies between 40 to 110 sec were observed after PTZ given i.p. in our experiments.

Members of the diterpene family have a controversial effect after administration of $GABA_A$ receptor antagonists. Whereas, forskolin, has been reported to prevent PTZ-induced seizures in mice (1) and it protects against bicuculline-induced convulsions (3); in hippocampal slices, it appears to enhance the generation of afterdischarges and therefore to be proconvulsant (5). In addition, sclareol glycol, another diterpene of the labdane family, appears to potentiate PTZ-induced seizures (20). Whether KA acts as activator of adenylate cyclase, similar to forskolin, remains to be investigated.

In our laboratory KA has shown hypotensive effects that are associated with the generation of nitric oxide (NO). Indeed, spontaneously hypertensive rats treated with KA, 20 mg/kg, in the presence and absence of L-N^G-nitroarginine methyl ester (L-NAME), a NO synthase (NOS) inhibitor,

the vaso-relaxant effects were suggested to be a NO-mediated event (21). It is not feasible that KA may be acting as anticonvulsant by a NO generating mechanism, because the doses needed to produce this effect are 10-fold higher than its anticonvulsant dose. In addition, the role of NOS inhibitors in seizure activity has a large variation (22). For instance, the model of PTZ-induce seizure in mice: it is inhibited by L-NAME (23); but it is neither affected by 7-nitroindazole, a preferential inhibitor of neuronal NOS nor by N^G-nitro-L-arginine, an arginine analogue (22).

A possible effect of KA on sodium channel acting by a mechanism similar to carbamazepine and phenytoin could not be ruled out, but these agents are ineffective (17) or tend to aggravate PTZ-induced absence seizure (14), on the contrary, valproic acid (16, 17) and KA are effective against PTZ-induced clonic-tonic seizures.

In conclusion, this work presents evidence that KA, at a dose relatively low (ED50 of 2.5 mg/kg), has anticonvulsant effects on the SSSC model, with a pattern of muscle contractions similar to valproic acid, but more potent than carbamazepine, phenytoin and valproic acid. Furthermore, it shows that KA is effective for decreasing PTZ-induced clonic-tonic seizures, as it was also reported for valproic acid (16). We do not have yet an explanation for the mechanism of action of KA, but these findings suggest that KA deserves attention and it should be tested in other animal models of seizures in order to fully characterize its anticonvulsant antivity.

ACKNOWLEDGMENTS

This work was supported by the Research Council (CDCHT) of the Universidad Centroccidental Lisandro Alvarado (016-ME-2001). It was presented in an abstract form at The Annual Meeting of Society for Neurosciences, Program No 94.19, 2005.

REFERENCES

1. **Sano M, Seto-Ohshima A, Mizutani A**. Forskolin suppresses seizure induced by pentylenetetrazol in mice. Experientia 1984; 40:1271-1272.
2. **Seamon KB, Padgett W, Daly JW**. Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc Natl Acad Sci 1981; 78: 3363-3367.
3. **Georgieva J**. Effect of diterpene sclareol glycol on convulsive seizures. Methods Find Exp Clin Pharmacol 1989; 11:335-340.
4. **Ameri A, Gleitz J, Peter T**. Bicuculline-induced epileptiform activity in rat hippocampal slices: suppression by Aconitum alkaloids. Planta Med 1997; 63: 228-232.
5. **Higashima M, Ohno K, Koshino Y.** Cyclic AMP-mediated modulation of epileptiform afterdischarge generation in rat hippocampal slices. Brain Res 2002; 949: 157-161.
6. **Usubillaga A, Capra MC**. Chemical constituents of *Espeletia semiglobulata*. Fitoterapia 1988; 59:382-384.
7. **Usubillaga A, Romero M, Aparicio R.** Kaurenic acid in Espeletiinae. Acta Hortic 2003; 597: 129-130.
8. **Davino SC, Giesbrecht AM, Roque NF.** Antimicrobial activity of kaurenoic acid derivatives substituted on carbon-15 Braz J Med Biol Res 1989; 22:1127-1129.
9. **Ohkoshi E, Makino M, Fujimoto Y.** Studies on the constituents of *Mikania hirsutissima* (Compositae). Chem Pharm Bull (Tokyo), 1999; 47: 1436-1438.
10. **Schwaiger S, Adams M, Seger C, Elimerer EP, Bauer R, Stuppner H**. New constituents of *Leontopodium alpinum* and their in vitro leukotriene biosynthesis inhibitory activity. Planta Med 2004; 70:978-985.
11. **Daló NL, Larson AA.** Spinal seizures evoked by sudden cooling of amphibian

isolated spinal cords: involvement of excitatory amino acids. Cryobiology 1991; 28: 255-267.
12. **Daló NL, Bracho GA, Piña-Crespo JC**. Motor impairment and neuronal damage following hypothermia in tropical amphibians. Int J Exp Pathol 2007; 88(1):1-7.
13. **Piña-Crespo JC, Daló NL**. Activity of common anticonvulsant drugs on spinal seizure-induced by sudden cooling. Prog Neuro-Psychopharmacol Biol Psychiatry 2006; 30:1202-1208.
14. **Cortez MA, Snead OC III**. Pharmacologic models of generalized absence seizures in rodents. In: Pitkanen A, Schwartzkroin PA, Moshe SL, Eds. Model of seizures and epilepsy, Elsevier Inc, 2006, p 111-126.
15. **Koella WP**. Animal experimental models in the study of antiepileptic drugs. In. Frey HH, Yanz D, Eds. Handbook of Experimental Pharmacology, Springer, Berlin, 1985; 74: p 281-339.
16. **Fisher RS**. Animal models of the epilepsies. In: Fisher RS, Coyle JC, Eds. Neurotransmitter and Epilepsy, Wiley-Liss; 1991, p 61-76.
17. **Loscher W, Honack D, Fassbender P, Nolting B**. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentyllenetetrazole seizure models. Epilepsy Res 1991; 8:171-189.
18. **Daló NL, Hackman JC, Davidoff RA**. Motoneuron depolarization, paroxysmal activity and reflex changes induced by rapid cooling of toad spinal cord. Comp Biochem Physiol 1995; 112A:517-525.
19. **Daló NL, Piña-Crespo JC**. Ketamine abolishes the tonic phase of the seizures evoked by sudden cooling of toad isolated spinal cords. Neuropharmacology 1992; 31:509-512.
20. **Georgieva J, Danchev N**. The effect of diterpene sclareol on seizure do not depend on central benzodiazepine receptors. Methods Find Exp Clin Pharmacol 1990; 12: 679-683.
21. **Neira N, Usubillaga A, Linarez G, Escobar A. Mujica F, Testa M, Sosa-Sequera M**. Vasorelaxant effect of sodium kaurenate: a possible nitric oxide-releasing agent (Abstract). Biocell 2003; 27(Suppl. 3):392.
22. **Wojtal K, Gniatkowska-Nowakowska A, Czuczwar SJ**. Is nitric oxide involved in the anticonvulsant action of antiepileptic drugs? Polish J. Pharmacol 2003; 55: 535-542.
23. **Kuputlu I, Uzbay T.** L-NAME inhibits pentylenetetrazol and strychnine-induced seizures in mice. Brain Res 1997; 753:98-101.