


Original Article

# Effect of intracerebroventricular administration of ascorbic acid on a seizure model induced by pentylenetetrazol in male rats

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## Abstract

**Introduction:** Epilepsy is one of the most common and chronic neurological disorders. It appears periodically and usually is concomitant with unpredictable seizures due to abnormal discharge of brain neurons. In this study, we investigated the anticonvulsant effect of ascorbic acid (AA) on seizures induced by pentylenetetrazole (PTZ) in male rats.

**Methods:** In this study PTZ (37 mg/kg) was injected every other day to induce kindling in male rats. AA (12.5, 25 and 50 mg/kg) was administered into the right lateral ventricle 30 minute before every PTZ injection. The seizure parameters were measured during 30 min after PTZ injection.

**Results:** Administration of 12.5 mg/kg of AA increased stage 4 latency compared to vehicle group. Conversely, 50 mg/kg of AA decreased stage 1 and 2 latency, increased stage 5 duration and decreased number of PTZ injections needed to achieve stage 5 seizure compared to vehicle treated animals.

**Conclusion:** It seems that the AA has dual effects on seizure parameters induced by PTZ. Low doses (12.5 mg/kg) have protective effects while high doses (50 mg/kg) have proconvulsant effects on seizure.

## Keywords:

Ascorbic acid;  
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Pentylenetetrazole;  
Rat

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## Introduction

Epilepsy is one of the most common diseases in nervous system (Hauser et al., 1991). The purpose of the treatment in patients with seizure is preventing epileptic seizures and the onset of seizures. There is no definitive treatment for this neurological disorder

and also around 30% of patients are drug resistance (Alimohammadi et al., 2014; Sofiabadi et al., 2012). Natural products and vitamins may helpful as add on therapies in epilepsy.

Vitamin C (L-ascorbate) is required for living organisms. This vitamin, as an antioxidant, protects organisms from oxidative stress (Padayatty et al., 2003). Interestingly recent reports demonstrated that

ascorbic acid (AA; a form of vitamin C) can increase the level of progesterone (Gonzalez-Ramirez et al., 2010). Progesterone has known antiepileptic properties. It has been observed that in some seizure models (seizures induced by pilocarpine) antioxidants such as melatonin, vitamin E and C have anticonvulsant effects (Sayyah et al., 2002); however it has recently been reported that plant compounds with an antioxidant effect do not increase the latency to myoclonic, clonic and tonic seizures in animal models of acute seizures (Xu and Stringer, 2008). AA is present at high concentrations and heterogeneous distribution in the mammalian brain. Previous studies have shown that various neurotransmitters such as glutamate, acetylcholine and dopamine are involved in the AA release from nerve terminals (Rebec and Pierce, 1994). These neurotransmitters are also involved in epilepsy (Kalichman, 1982). On the other hand, it has been shown that low doses of AA has agonistic effects on glutamatergic and dopaminergic systems while, in high doses has antagonistic effects (Rebec and Pierce, 1994).

Considering the above literature, we hypothesized that AA may have complicated effects on epilepsy. Therefore, in this study we investigated the effect of low and high doses of AA on seizures induced by pentylentetrazole (PTZ) in male rats.

## Materials and methods

### Animals

Male rats, weighing 200–250 g, were obtained from the animal house at Sabzevar University of Medical Sciences. After three handling days, four groups (10 each) were randomly selected. Rats were placed in polypropylene cages with paddy husk as bedding. The animals were housed at a temperature of  $23 \pm 2$  °C and relative humidity of 30–70%. All procedures involving the care and use of the animals were conducted in accordance with the Sabzevar University Ethics Committee. All experiments were completed at the same time (8.00 am to 2.00 pm) to avoid the bias of circadian rhythms. After a 3-day period of handling, four groups (each group of 10 rat) are randomly selected

### Surgical procedure

For stereotaxic surgery, the animals were anesthetized by ketamine (100 mg/kg, ip). The

animals were implanted with a 22-gauge guide cannula in the right lateral brain ventricular (coordinates: A, -0.8 mm; L, +1.6 and 4.3 mm below dura) (Paxinos and Watson, 1986). Injection of AA into the lateral ventricle was performed using 27-gauge cannula. In order to place the injection cannula in the desired location, the cannula during surgical procedure is located one millimeter above the target area and is fixed by dental cement.

### Drug injection

AA was dissolved in normal saline and pH adjusted to 7.3–7.4 using 1N NaOH. The solution was sterilized through microfilters (0.2µm, Minisart, NML, Sartorius, Germany).

Drug were infused (0.5µl over 2 min) via a 27-gauge cannula, which was 1mm below the tip of 22-gauge cannula. In the first group, 30 minutes after microinjection of saline into the lateral ventricle of the brain, PTZ (37 mg/kg) (Arash et al., 2013) was injected (ip). This process is repeated every 48 hours until stage 5 of seizure occur.

Immediately after injection of PTZ, the rats were transported to a separate cage and the seizure stages are measured during 30 minutes. These stages are progressively replicated by injection until stage 5 of seizure occur (the last stage of the Racine score). In other words, in the first injections stage, 1 to 2 and in subsequent injections, a higher degree of seizure stages occurs. These steps include: stage zero, without any response; stage one, muscle contractions of the face, ears and whiskers; stage 2, spreading seizure wave around the body without standing; stage 3, standing on rearing of both legs with anterior limb clonus; stage 4, tonic and clonic seizures and loss of balance and stage 5, tonic and clonic seizures of the body and loss of balance and falling (Racine, 1972). The measurable and comparable parameters in this study were: delay time to stage1 (stage 1 latency, S1L), to stage 2 (stage 2 latency, S2L) and to stage 4 seizures (stage 4 latency, S4L), the duration of the stage 5 (S5D) and the number of injections needed to reach stage 5 seizure.

In the second to fourth groups, doses of AA (12.5, 25 and 50 mg/kg) (Kim et al., 2016; Santos et al., 2008) was injected intracerebroventricularly (ICV). In all groups, immediately after each PTZ injection, the animal's behavior was monitored during 30 minutes.

Injection of AA and PTZ continued until stage 5 seizures were elicited.

### Statistical analysis

Results obtained are expressed as the means $\pm$ SEM and accompanied by the number of observations. Comparison of data from animals receiving AA with those receiving saline was carried out by Student's t-test. A *P* value less than 0.05 was considered to represent a significant difference.

## Results

All kindled rats responded with stable stage 5 seizures in either a noninfusion condition or after normal saline injection. At the doses employed, AA did not exert any noticeable effect on behavioral or locomotor activity. Histological assessment indicated that the infusion cannula were positioned in the lateral ventricle of the brain.

### The effect of AA on stage 1 latency

Statistical analysis of the data showed that ICV injection of AA (50 mg/kg) decreased significantly in the latency to the onset of stage 1 seizure when compared to the vehicle group (*P*<0.05), but lower doses had (25 and 12.5 mg/kg) no significant effect on this parameter (Fig. 1A).

### The effect of AA on stage 2 latency

Data analysis showed that ICV injection of AA (50 mg/kg) significantly decreased latency to the onset of stage 2 seizure when compared to the vehicle group (*P*<0.05), but lower doses (25 and 12.5 mg/kg) had no significant effect on this parameter (Fig. 1B).

### The effect of AA on stage 4 latency

ICV injection of AA had dual effect on delayed time to reach stage 4 seizure (stage 4 latency). Injection of 12.5 mg/kg resulted in a significant increase (*P*<0.05) in the latency to the onset of stage 4 seizure; however, injection of 25 mg/kg of AA reduced the latency to the onset of stage 4 seizure (*P*<0.05, Fig. 1C).

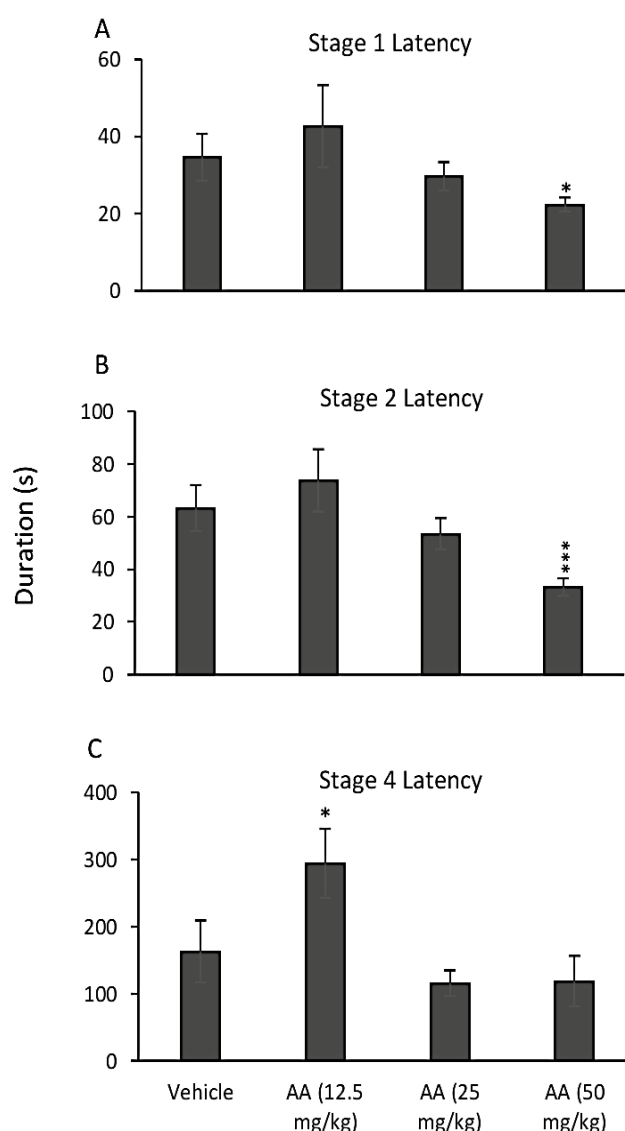
### The effect of AA on stage 5 duration

ICV injection of different doses of AA on the duration of stage 5 seizures showed that as the dose enhancement, the duration of stage 5 of seizure increased, so that in the group receiving a dose of

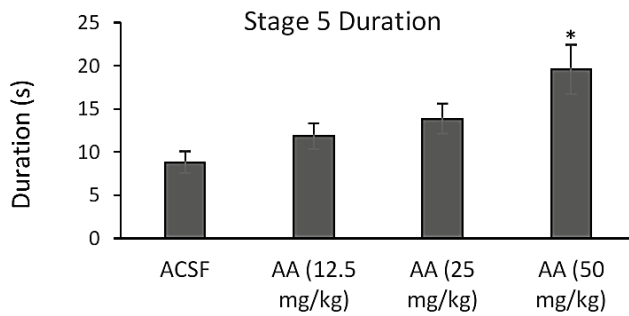
12.5 mg/kg of AA, there was a significant difference between the duration of stage 5 and vehicle group (*P*<0.05, Fig. 2).

### The effect of AA on the number of injections needed to reach stage 5 seizure

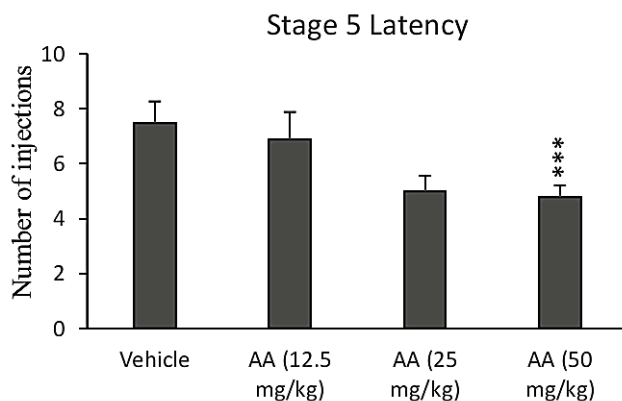
The number of PTZ injections required to reach the stage 5 seizure was another parameter that was analyzed in this study. The results showed that by increasing the dose of AA, the number of injections needed to reach stage 5 of the seizure decreased. In the group receiving 50 mg/kg, the number of PTZ injections to reach phase 5 was significantly lower than that of the vehicle group (*P*<0.05, Fig. 3).



**Fig.1.** Effect of ascorbic acid on stage 1 latency (A), stage 2 latency (B) and stage 4 latency (C) in kindled rats induced by PTZ. \**P*<0.05 when compared to the vehicle group by two-tailed paired t-test; n=10.



**Fig.2.** Effect of ascorbic acid on stage 5 duration in kindled rats induced by PTZ. \* $P < 0.05$  when compared to the vehicle group by two-tailed paired t-test;  $n = 10$ .



**Fig.3.** Effect of ascorbic acid on the number of injections needed to reach stage 5 seizure in kindled rats induced by PTZ. \*\*\* $P < 0.001$  when compared to the vehicle group;  $n = 10$ .

## Discussion

Results of the present study showed that ICV injection of AA can exert a dual effect on seizures elicited by PTZ. AA in high dosage (50 mg/kg) had proconvulsant and in lower dosage (12.5 and 25 mg/kg) had anticonvulsant effect somewhat.

In a study about the effect of AA (median consumption) on spatial learning of mice, it has been shown that learning decreased compared to control group. Also, in mice that have reached the learning boundary, working memory and the spatial reference was less than that of the control group (Nasri et al., 2008). In this regard, another study has shown that AA can reduce (directly or through the effects of other neurotransmitters) learning and spatial memory after injection into the lateral ventricles (McNamara and Skelton, 1993).

In previous studies, the protective effects of AA on seizure have been investigated. The combination of low intensity exercise and AA has been shown to

decrease the seizure induced by kainic acid in mice (Kim et al., 2016). It has also been shown that decreasing of AA in the brain increase susceptibility to seizure (Warner et al., 2015). A study by Gonzalez-Ramirez et al. has been shown that AA increased the progesterone concentration by 531% and 253% after 30 minutes, and 24 hours (respectively) compared to normal. Therefore, AA, along with progesterone, increases the latency in the onset of myoclonic, tonic and clonic induced by PTZ and reduces the prevalence of clonic and tonic seizures (Gonzalez-Ramirez et al., 2010). In the current study it seems that AA in high dosage (50 mg/kg) increased progesterone; however another mechanism (such as NMDA receptors and its current; see later paragraph) were dominant. The lower dosage which elicited anticonvulsant effect somewhat NMDA current has not prominent.

Regarding to neuroprotective mechanism of AA, reported that AA at a dose of 250 mg/kg increased the latency for primary seizures and reduced the death rate of pilocarpine seizures. Also, AA pre-treatment reduced the level of lipid peroxidase. The results showed that the protective effect of AA in young mice was due to decreased lipid peroxidase levels and increased catalase activity after seizures (Santos et al., 2008). Another study by Esmaili et al. showed that AA at a dose of 100 to 500 mg/kg improve spatial learning in a dose-dependent manner, but injection of 1000 mg/kg causes a significant reduction in spatial learning (Esmaili et al., 2003). Considering the similarity of mechanisms involved in seizure and memory, it seems that the effect of different doses of AA on seizures is justified. As with memory-learning studies (Esmaili et al., 2003), this compound has proconvulsant effect at high doses and anticonvulsant at lower doses.

AA, by antagonizing NMDA receptors, produces protective effects in the nervous system. Of course, this effect is observed at millimolar concentrations. This amount of concentration has been reported under conditions such as severe brain trauma. Also, in conditions such as ischemia and seizure activity, an increase in the AA content of six to eight times is reported (Cammack et al., 1992; Rebec and Pierce, 1994). In this regard it has been shown that perfusion of the cells with high concentrations of ascorbate (1-3 mM) rapidly and reversibly attenuated NMDA-induced inward currents (Majewska et al., 1990). It



seems microinjection of AA in 50 mg/kg thirty minute before PTZ injection (every other day, 15 times) increase its concentration in the brain and attenuate NMDA-induced inward currents. The result of this decline is up-regulation of NMDA receptors and during seizure propagation up-regulated NMDA receptors potentiate seizure waves and seizure behavior. Similar to this result, it has been shown that chronic supplementation of vitamin C at a dose of 60 mg/day in natural development period leads to its prooxidative results to brains in observed animals (Monte-Guedes et al., 2011).

Concerning the low doses of AA in this study, we think up-regulation does not occur and maybe the microinjected AA and the endogenous ascorbate decrease NMDA currents and finally prevent seizure propagation. On the other hand, as it mentioned before ascorbic acid is exogenous powerful antioxidant molecules that act together with other endogenous antioxidant systems within tissue cells in order to scavenge the formed reactive oxygen species (Jakeman and Maxwell, 1993). In addition, it is likely to be part of the anticonvulsant effect of vitamin C in preventing the accumulation of unsaturated fatty acids in the brain during seizure (Santos et al., 2008). Thus may be one of these mechanism or all of them involved in anticonvulsant effect of AA. Further studies are needed to fully clarify the anticonvulsant mechanisms of ascorbic acid.

## Conclusion

The results of this study showed that the effect of AA on seizure is dose-dependent. High doses of this vitamin, as it impairs learning and memory, exacerbates seizures and low doses of it prevent the progression of seizures. Based on this study, the similarity of the results with memory studies and learning the authenticity of the similarity of mechanisms involved in memory and seizure is reaffirmed and emphasized.

## Acknowledgments

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## Conflict of interest

The authors have no conflict of interests to declare.

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