ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research Paper Acta Neurobiol Exp 2015, 75: 192–199

# Long-term ascorbic acid administration causes anticonvulsant activity during moderate and long-duration swimming exercise in experimental epilepsy

**Erkut Tutkun[1], Gokhan Arslan[2], Recep Soslu[1], Mustafa Ayyildiz[2], and Erdal Agar[2]***

[1]Faculty of Yasar Dogu Sport Sciences, [2]Department of Physiology, Faculty of Medicine, University of Ondokuz Mayis, Samsun, Turkey, *Email: eragar@omu.edu.tr

The benefits of regular exercise on brain health are undeniable. Long-term exercise increases the production of reactive oxygen species in brain. Therefore, athletes often consume antioxidant supplements to remedy exercise-related damage and fatigue during exercise. The aim of this study is to evaluate the role of ascorbic acid in the effects of different intensities of swimming exercise on the brain susceptibility to experimental epilepsy in rats. Ascorbic acid was administered intraperitoneally (ip) during three different swimming exercise programme for 90 days (15 min, 30 min, 90 min/day). The anticonvulsant activity regarding the frequency of epileptiform activity appeared in the 80 min after 500 units intracortical penicillin injection in 30 min and 90 min/day exercise groups. The administration of ascorbic acid (100 mg/kg, ip) did not alter the anticonvulsant properties seen in the in short-duration (15 min/day) swimming exercise group. The amplitude of epileptiform activity also became significant in the 110 and 120 min after penicillin injection in the moderate (30 min/day) and long duration (60 min/day) groups, respectively. The results of the present study provide electrophysiologic evidence that long-term administration of ascorbic acid causes anticonvulsant activities in the moderate and long-duration swimming exercise. Antioxidant supplementation such as ascorbic acid might be suggested for moderate and long-duration swimming exercise in epilepsy.

Key words: ascorbic acid, epilepsy, swimming exercise, vitamin

## INTRODUCTION

Ascorbic acid (vitamin C) is probably the most important water-soluble antioxidant, which involved in a number of biochemical pathways that are important metabolism and health of exercising individuals (Peake 2003, Rosa et al. 2009). Several studies examined the effects of ascorbic acid supplementation on the changes in amount of antioxidants in blood and performance in exercise (Clarkson and Thomson 2000, Urso and Clarkson 2003). Contradictory roles for ascorbic acid in the various kind of exercise have been suggested either beneficial adverse (Evans 2000, Jourkesh et al. 2007) or no effect (Nieman et al. 2002) or deleterious effects (Gomez-Cabrera et al. 2008).

*Correspondence should be addressed to E. Agar*
*Email: eragar@omu.edu.tr*

*Received 05 December 2014, accepted 19 June 2015*

On the other hand, ascorbic acid and physical exercise have separately neuroprotective properties in reducing the frequency and severity of seizures in several models of experimental epilepsy (Arida et al. 1998, 2004, Setkowicz and Mazur 2006, Ayyildiz et al. 2007, Souza et al. 2009, Tutkun et al. 2010). Ascorbic acid has been found to be effective against pentylenetetrazol (PTZ)-induced seizures and penicillin-induced seizures (Oliveria et al. 2004, Yildirim et al. 2010). In addition, pretreatment with ascorbic acid for 5 days, at a dose of 500 mg/kg, produced a neuronal protection against pentylenetetrazole-induced seizure by reducing onset to myoclonic, clonic and tonic seizures in immature rats (González-Ramírez et al. 2010). However, chronic administration of ascorbic acid, at a dose of 60 mg/kg, may have a pro-oxidant activity in the developing animals' brain (Monte-Guedes al. 2011). Epileptics have been discouraged

from participation in a regular physical activity for the possibility of inducing or increasing seizure activity (Denio et al. 1989, Nakken et al. 1990). But most of studies from adult animals have demonstrated that swimming exercise decreased the susceptibility to subsequent pilocarpine-induced seizure (Setkowicz and Mazur 2006), pentylenetetrazole-induced seizure (Souza et al. 2009) and penicillin-induced epileptiform activity in male rats (Tutkun et al. 2010). However, moderate (30 min per day) and long-duration (60 min per day) swimming exercise were not effective in reducing either the frequency or severity of seizure (Tutkun et al. 2010).

Contradictory roles have been suggested for the chronic use of ascorbic acid in both the various kind of exercise and brain. Therefore, in the present study, the role of intraperitoneal administration of ascorbic acid in the impact of different intensities of swimming exercise on the brain susceptibility to penicillin-induced epileptic activity was determined in rats.

## METHODS

Adult male Wistar rats weighing 190–240 g (Ondokuz Mayis University of Turkey) were used throughout this study after at least 1 week of acclimatization. All described procedures were approved by the local ethics committee. All animals were kept in a temperature controlled (22±1ºC) environment on a 12-h light/dark cycle. Rats were assigned to the following experiments and groups: intracortical (ic) delivery of (Group 1) 2.5 µl artificial cerebrospinal fluid [aCSF containing (mM): NaCl, 124; KCl, 5; $KH_2PO_4$, 1.2; $CaCl_2$, 2.4; $MgSO_4$, 1.3; $NaHCO_3$, 26; glucose, 10; HEPES, 10; pH 7.4 when saturated with 95% $O_2$ and 5% $CO_2$] (ic); (Group 2, Control group) 500 units penicillin (2.5 µl, ic); (Group 3) 15 minutes-trained for 90 days+500 units penicillin (2.5 µl, ic) + ascorbic acid (100 mg/kg, ip); (Group 4) 30 minutes-trained for 90 days+500 units penicillin (2.5 µl, ic)+ascorbic acid (100 mg/kg, ip); (Group 5) 60 minutes-trained for 90 days+500 units penicillin (2.5 µl, ic)+ascorbic acid (100 mg/kg, ip); (Group 6, Sham group) adapted to the water+500 units penicillin (2.5 µl, ic)+ascorbic acid (100 mg/kg, ip). Each animal group was composed of seven rats.

### Adaptation to the water

All animals were adapted to the water before the beginning of the experiment. The rats were kept in shallow water at 32°C for seven days/week, from 10:00 AM to 12:00 AM for adaptation. The adaptation to the water proceeded during experimental period. The purpose of the adaptation to water was to reduce stress without promoting a physical training adaptation (Souza et al. 2009, Tutkun et al. 2010).

### Exercise training program

The swimming performed in water at a temperature of 32–33°C between 10:00 AM–12:00 AM. The training period lasted 90 days and consisted of 15, 30, and 60 minutes daily sessions for seven days/week without workload. Exercise performed by swimming in two training glass tanks (length 100 cm, width 50 cm, depth 50 cm) containing tap water. After 90 days swimming, rats were prepared for induction of epileptiform activity in the next training time (Tutkun et al. 2010).

### Drug administration

L-Ascorbic acid (Sigma-Aldrich) (pH 7.0) was used in the experiments. Ascorbic acid was dissolved in sterile physiological saline solution. After adaptation period, animals received a daily dose of 100 mg/kg ascorbic acid intraperitoneally (ip) for 90 days. At the end of this period, ascorbic acid administration was stopped, and 24 h after the last administration of ascorbic acid, all rats were anesthetized for induction of epileptiform activity.

### Induction of epileptiform activity

The animals were anesthetized with urethane (1.25 g/kg, ip) and placed in a stereotaxic frame. Rectal temperature was maintained between 36 and 37°C using a feedback-controlled heating system. The left cerebral cortex was exposed by craniotomy (5 mm posterior to bregma and 3 mm lateral to sagittal sutures). The epileptic focus was produced by 500 units of penicillin G potassium injection (1 mm beneath the brain surface by a Hamilton microsyringe type 701N; infusion rate 0.5 µl/min) (Kozan et al. 2007, Aslan et al. 2009). Penicillin was prepared in the

sterile distilled water and administered intracortically in a volume of 2.5 µl.

### Electrocorticography recordings

Two Ag–AgCl ball electrodes were placed over the left somatomotor cortex (electrode coordinates: first electrode, 2 mm lateral to sagittal suture and 1 mm anterior to bregma; second electrode, 2 mm lateral to sagittal suture 5 mm posterior to bregma) (Cakil et al. 2011). The common reference electrode was fixed on the pinna. The electrocorticography (ECoG) activity was continuously monitored on a four-channel recorder (PowerLab, 4/SP, AD Instruments, Castle Hill, Australia). All recordings were made under anesthesia and stored on a computer (Kozan et al. 2006). The frequency and amplitude of epileptiform ECoG activity was analyzed off-line.

### Data analysis

All statistical procedures were performed using SPSS statistical software package (version: 13.0). The results are given as the means ± standard error of the mean (SEM). Data analysis was performed using one way ANOVA and Bonferroni post hoc tests for comparisons. Statistical significance was set at $P<0.05$.

## RESULTS

It is well known that the intracortical injection of penicillin induces epileptiform activity in rats (Arslan et al. 2013, 2014). The 500 units of penicillin were used to induce an epileptiform ECoG activity characterized by bilateral spikes and spike waves complexes in this study (Fig. 1A). The means of spike frequency and

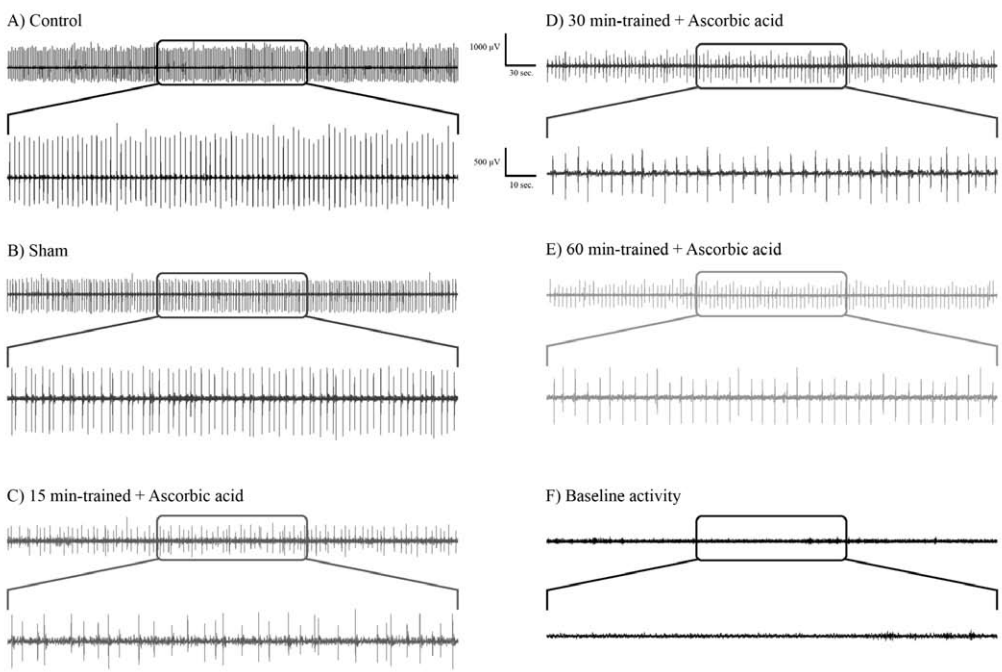


Fig. 1. (A) The intracortical injection of penicillin (500 IU) induced epileptiform activity on ECoG in adapted to water group (control group). (B) Administration of the ascorbic acid (100 mg/kg, per day for 90 days, ip) did not alter either the mean frequency or amplitude of penicillin-induced epileptiform activity in adapted water group (sham group). (C) Administration of the ascorbic acid (100 mg/kg, per day for 90 days, ip) did not alter either the mean frequency or amplitude of penicillin-induced epileptiform activity in 15 minutes-trained group (swimming exercise 15 min per day, for 90 days). (D) Administration of the ascorbic acid (100 mg/kg, per day for 90 days, ip) caused a decrease in the mean frequency or amplitude of penicillin-induced epileptiform activity in 30 minutes-trained group (swimming exercise 30 min per day, for 90 days). (E) Administration of the ascorbic acid (100 mg/kg, per day for 90 days, ip) also caused a decrease in the mean frequency or amplitude of penicillin-induced epileptiform activity in 60 minutes-trained group (swimming exercise 60 min per day, for 90 days). (F) Presents baseline ECoG activity before penicillin. Representative ECoGs are presented for the 120 minutes after penicillin administration.

amplitude were 35.7±4.6 spike/min and 1087±104 μV, respectively (Fig. 1A).

Figure 2 and 3 show the role of ascorbic acid in the effects of various intensities of swimming exercise on penicillin-induced epileptiform activity in rats. The effective dose of ascorbic acid (100 mg/kg, ip) was administered 30 min before swimming exercise for 90 days to all experimental groups. The mean frequency and amplitude of ECoG epileptiform activity did not change in the sham group (Figs 2, 3). The means of spike frequency and amplitude of epileptiform activity were 27.6±3.2 spike/min and 838±88 μV after 120 min from penicillin injection in shame group, respectively (Fig. 1B). However, the mean frequency of the spike activity decreased in the 60 min in group 3, in the 80 min in group 4 and in the 80 min in group 5 after penicillin injection (Fig. 2). In the presence of ascorbic acid, the mean amplitude of ECoG epileptiform activity became significant after 110 and 120 minutes penicillin injection in groups 4 and 5, respectively (Fig. 3). The mean spike frequency of epileptiform activity was 12.4±2.8, 16.7±3.7, 14.9±3.4 spike/min, and the mean amplitude was 517±94, 554±76, 603±83 μV after 120 minutes from penicillin injection in the group 3, group 4, group 5 swimming training groups, respectively (Fig. 1 C–E). The intracortical injection of aCSF (2.5 μl), did not cause any change in the frequency or amplitude of ECoG activity with respect to the control baseline in the non-penicillin injected animals (Fig. 1F).

## DISCUSSION

Exercise can produce an imbalance between reactive oxygen species and antioxidants, which is referred to as oxidative stress (Urso and Clarkson 2003). Ascorbic acid is water-soluble antioxidant in the brain extracellular fluid (Niki 1991). It has been suggested

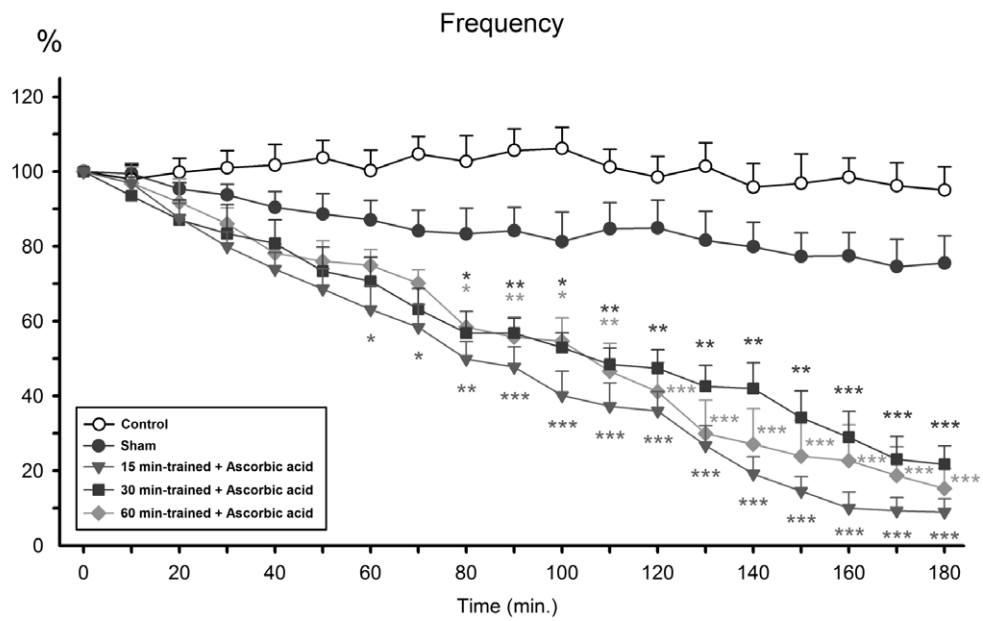


Fig. 2. The effects of ascorbic acid (100 mg/kg, per day for 90 days, ip) on the mean spike frequency of penicillin-induced epileptiform ECoG activity in swimming exercised groups. The administration of ascorbic acid, at a dose of 100 mg/kg (ip), caused a significant decrease in the mean frequency of ECoG epileptiform activity 80 minutes after penicillin injection in the 30 and 60 minutes swimming training groups. The administration of ascorbic acid did not cause an additional anticonvulsant activity in 15 minutes swimming training group. The short-duration swimming exercise decreased the frequency of epileptiform activity in the mean frequency of ECoG epileptiform activity 80 minutes after penicillin the mean frequency of ECoG epileptiform activity 60 minutes after penicillin injection in the 15 minutes swimming training group. **P*<0.05; ***P*<0.01; ****P*<0.001 indicate significant differences compared to sham group. The percentage frequency of epileptiform ECoG activity value depends on the frequency of epileptiform ECoG activity before and after the substance administered and is defined as:

$$\text{Frequency Value \%} = \frac{\text{The mean of spike frequency after substance administered}}{\text{The mean of spike frequency before substance administered}} \times 100$$

that ascorbic acid has neuro-protective properties against exercise induced damage and neurological disorders including epilepsy (Clarkson and Thompson 2000, Hamed and Abdullah 2004). The present study demonstrated that administration of ascorbic acid, at a dose of 100 mg/kg/day for 90 days caused the anticonvulsant activity in moderate duration and long-duration long term swimming exercise groups against penicillin-induced epileptiform activity.

Several studies have been performed to answer the question whether supplementation of ascorbic acid has beneficial effect on exercise. The administration of ascorbic acid as single dose of 1 000 mg (2 hours before 90 min shuttle run) increased the plasma level of vitamin C but did not alter the development of muscle soreness after exercise (Thompson et al. 2001). The consumption of ascorbic acid, at a dose of 1 000 mg/day for 12 weeks, did not affect air force officers' performance in the walk/run field test (Gey et al. 1970). Moreover, the administration of ascorbic acid (500 mg/kg) significantly hampered endurance capacity in rats and did not improve $VO_2$ max associated with training in rats and in humans (Gomez-Cabrera et al. 2008). In contrast, Jakeman and Maxwell (1993) found that the administration of ascorbic acid, at a dose of 400 mg/day, for 21 days prior to and 7 days after performing 60 min of box-stepping exercise, showed a protective effect against eccentric exercise-induced muscle damage. The administration of ascorbic acid showed beneficial effects against exhaustive swimming and high altitude-associated lung injury in rats (Al-Hashem 2012).

On the other hand, ascorbic acid has showed neuroprotective properties in variety of experimental epilepsy models (Oliveira et al. 2004, Yildirim et al. 2010). A synthetic derivative of ascorbate (α-tocopheryl-L-ascorbate-2-O-phosphate diester potassium salt) prevented the occurrence of ferric ion–induced epileptic discharges in a rat model of post-traumatic epilepsy (Yamamoto et al. 2002). Ascorbate, at a dose of 300 mg/kg, protected against PTZ-induced convulsions, whereas ascorbate at a dose of 30 mg/kg, did not change PTZ-induced convulsions (Oliveira et al. 2004). In our previous studies, ascor-

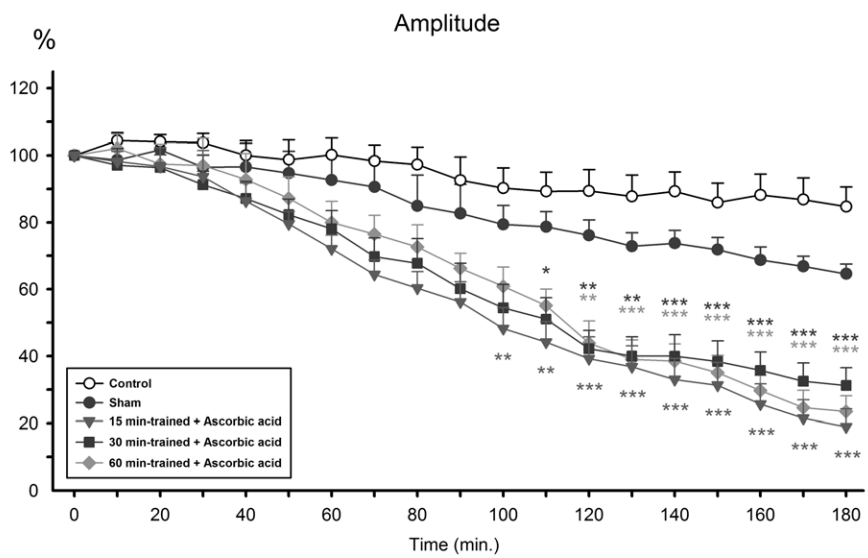


Fig. 3. The effects of ascorbic acid (100 mg/kg, per day for 90 days, ip) on the mean spike amplitude of penicillin-induced epileptiform ECoG activity in swimming exercised groups. The administration of ascorbic acid, at a dose of 100 mg/kg (ip), caused a significant decrease in the mean amplitude of ECoG epileptiform activity 110 and 120 minutes after penicillin injection in the 30 and 60 minutes swimming training groups, respectively. Short duration swimming training decreased the amplitude of ECoG activity 100 minutes after penicillin injection in the 15 minutes swimming training group. **P*<0.05; ***P*<0.01; ****P*<0.001 indicate significant differences compared to sham group. The percentage amplitude of epileptiform ECoG activity value depends on the amplitude of epileptiform ECoG activity before and after the substance administered and is defined as:

$$\text{Amplitude Value \%} = \frac{\text{The mean of spike amplitude after substance administered}}{\text{The mean of spike amplitude before substance administered}} \times 100$$

bic acid, at doses of 50, 100, 200 and 400 mg/kg was effective in decreasing the frequency of penicillin-induced epileptiform activity in rats (Ayyildiz et al. 2007, Yildirim et al. 2010). Although a single dose of ascorbic acid (100 mg/kg) decreased the frequency of epileptiform activity in rat (Ayyildiz et al. 2007, Yildirim et al. 2010), pretreatment with ascorbic acid, at a dose of 100 mg/kg, for 7 days did not change the latency and frequency of epileptiform activity (Ayyildiz et al. 2007). Conversely, ascorbate, at a dose of 100 mg/kg, potentiated the duration of convulsive episodes (Oliveira et al. 2004) and ascorbate, at doses of 50-200 mg/kg, enhanced amphetamine-induced behaviour activation (Wambebe and Sokomba 1986) and conditioned place preference (Pierce et al. 1995). Mendes-da-Silva and coauthors (2014) suggested that ascorbic acid, a low dose (30 mg/kg/d for 22 days) acted as an antioxidant whereas high doses (60, 120 mg/kg/d for 22 days) acted as a pro-oxidant, as indicated by cortical spreading depression propagation and malondialdehyde levels in developing rats. Chronic administration of ascorbic acid (60 mg/kg/d for 21 days) acted as a pro-oxidant in developing animals by enhancing cortical spreading depression (Monte-Guedes et al. 2011). Furthermore, ascorbate, at a high dose of 500 mg/kg, had either no or opposing effect on these behaviour (Tolbert et al. 1979, Pierce et al. 1995) suggesting a biphasic effect of ascorbate on the different functions of nervous systems. Apparently, there is a striking disagreement among studies regarding the effect of long-term ascorbic acid supplementation on exercise or various pathological situations, suggesting either an antioxidant or a pro-oxidant or no effect, depending on experimental produces, dose of ascorbic acid, animal species, age of animals and exercise models applied (Paschalis et al. 2014, Sawicka-Glazer and Czuczwar 2014). However, in the present study, long-term administration of ascorbic acid (100 mg/kg/d for 90 days) did not cause any change in either frequency or amplitude of penicillin-induced epileptiform activity in non-exercised group. This result shows that chronic administration of ascorbic acid, at a 100 mg/kg, causes neither anticonvulsant nor proconvulsant activity in adult rat.

Physical exercise also have neuro-protective role in reducing severity of seizures in epilepsy (Arida et al. 1998, 2004, Setkowicz and Mazur 2006, Souza et al. 2009, Tutkun et al. 2010). Although short duration (15 min per day for 90 days) swimming exercise reduced the frequency and amplitude of epileptic activity, moderate-duration (30 min per day for 90 days) and long-duration (60 min per day for 90 days) swimming exercise did not alter either the frequency or amplitude of epileptiform activity (Tutkun et al. 2010). For the first time, in the present study, the effective dose of ascorbic acid (100 mg/kg, ip) on different intensities of swimming exercise was evaluated in penicillin-induced epileptic rats. The administration of ascorbic acid (100 mg/kg, ip for 90 days) did not alter the appearance time of anticonvulsant activity in the short duration (15 min per day for 90 days) swimming exercise group compare to swimming group without ascorbic acid administration (Tutkun et al. 2010). However, the administration of ascorbic acid (100 mg/kg, ip for 90 days) caused anticonvulsant activity in the 80 min from the penicillin injection in moderate-duration (30 min per day for 90 days) and long-duration (60 min per day for 90 days) swimming exercise groups. This point may be considered interesting, since either chronic administration of ascorbic acid or moderate and long-duration swimming exercise did not cause an anticonvulsant activity in penicillin-induced epileptiform activity in previous studies (Ayyildiz et al. 2007, Tutkun et al. 2010). The long-duration of physical exercise may produce different effects on the brain function, since it is well established that physical exercises are able to alter the release of neurotrophins, neurotransmitters, free radicals and neuropeptides in different brain areas, which are involved in epilepsy (Chaouloff et al. 1987, Neeper et al. 1995, Vissing et al. 1996, Arida et al. 2009). As illustrated in the present study, the long-term administration of ascorbic acid may cause the appearance of beneficial effects of swimming by increasing antioxidant defences and reducing the basal production of oxidants in moderate and long-duration swimming exercise. If long-term exercise causes the creation of oxygen free radicals (Kwon et al. 2014, Paschalis et al. 2014) and ascorbic acid is probably the most important water-soluble antioxidant (Peake 2003, Rosa et al. 2009) then ascorbic acid might demonstrate the beneficial effects of exercise due to its free radical scavenging activity. In addition, mild exercise causes the release of the brain-derived neurotrophic factor and the nerve growth factor, which play an anti-epileptic role in the different models of epilepsy (Takahashi et al. 1999, Vezzani et al. 1999). It is therefore logical to expect beneficial effects in cases of ascorbic acid supplementation during long-term exercise although there is disagreement regarding the effect of long-term supplementation of ascorbic acid on exercise or various pathological situations. The main limitation of present study was that all measurements were conducted by using only ECoG recording technique in the epilepsy setting. We have not determined the cer-

tain mechanism of these effects in the present study. Therefore, further biochemical and neurochemical investigations are required to explore certain mechanism involved in these effects.

## CONCLUSIONS

The results of present study show that long-term administration of ascorbic acid did not cause any change in the frequency and amplitude of penicillin-induced epileptiform activity in non-exercised animals. However, long term administration of ascorbic acid caused anticonvulsant activity in the moderate and long-duration swimming exercise. These findings suggest that long term antioxidant supplementation could have beneficial effects during both moderate and long-duration exercise in epilepsy.

## REFERENCES

Al-Hashem FH (2012) Potential roles for vitamins E and C in combination in modulating exhaustive swimming and high altitude-associated lung injury in rats. Saudi Med J 33(4): 367-374.

Arida RM, de Jesus Vieira A, Cavalheiro EA (1998) Effect of physical exercise on kindling development. Epilepsy Res 30: 127–132.

Arida RM, Scorza FA, Terra VC, Cysneiros RM, Cavalheiro EA (2009) Physical exercise in rats with epilepsy is protective against seizures: evidence of animal studies. Arq Neuropsiquiatr 67: 1013–1016.

Arida RM, Sanabria ER, da Silva AC, Faria LC, Scorza FA, Cavalheiro EA (2004) Physical training reverts hippocampal electrophysiological changes in rats submitted to the pilocarpine model of epilepsy. Physiol Behav 83: 165–171.

Arslan G, Alici SK, Ayyildiz M, Agar E (2013) The role of CB1-receptors in the proconvulsant effect of leptin on penicillin-induced epileptiform activity in rats. CNS Neurosci Ther 19: 222–228.

Arslan G, Ayyildiz M, Agar E (2014) The interaction between ghrelin and cannabinoid systems in penicillin-induced epileptiform activity in rats. Neuropeptides 48: 345–352.

Aslan A, Yildirim M, Ayyildiz M, Guven A, Agar E (2009) The role of nitric oxide in the inhibitory effect of ghrelin against penicillin-induced epileptiform activity in rat. Neuropeptides 43: 295–302.

Ayyildiz M, Coskun S, Yildirim M, Agar E (2007) The effect of ascorbic acid on penicillin-induced epileptiform activity in rats. Epilepsia 48: 1388–1395.

Cakil D, Yildirim M, Ayyildiz M, Agar E (2011) The effect of co-administration of the NMDA blocker with agonist and antagonist of CB1-receptor on penicillin-induced epileptiform activity in rats. Epilepsy Res 93: 128–137.

Chaouloff F, Laude D, Merino D, Serrurrier B, Guezennec Y, Elghozi JL (1987) Amphetamine and alpha-methyl-p-tyrosine affect the exercise-induced imbalance between the availability of tryptophan and synthesis of serotonin in the brain of the rat. Neuropharmacology 26: 1099–1106.

Clarkson PM, Thompson HS (2000) Antioxidants: what role do they play in physical activity and health? Am J Clin Nutr 72(2 Suppl): 637S–646S.

Denio LS, Drake ME Jr, Pakalnis A (1989) The effect of exercise on seizure frequency. J Med 20: 171–176.

Evans WJ (2000) Vitamin E, vitamin C, and exercise. Am J Clin Nutr 72(2 Suppl): 647S–652S.

Gey GO, Cooper KH, Bottenberg RA (1970) Effect of ascorbic acid on endurance performance and athletic injury. JAMA 211: 105.

Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J, Vina J (2008) Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87: 142–149.

González-Ramírez M, Razo-Juárez LI, Sauer-Ramírez JL, González-Trujano ME, Salgado-Ceballos H, Orozco-Suarez S (2010) Anticonvulsive effect of vitamin C on pentylenetetrazol-induced seizures in immature rats. Pharmacol Biochem Behav 97: 267–272.

Hamed S, Abdullah M (2004) Trace elements and electrolytes homeostasis and their relation to antioxidant enzyme activity in brain hyperexcitability of epileptic patients. Journal of Pharmacological Sciences 96: 349–359.

Jakeman P, Maxwell S (1993) Effect of antioxidant vitamin supplementation on muscle function after eccentric exercise. Eur J Appl Physiol Occup Physiol 67: 426–430.

Jourkesh M, Ostojic SM, Azarbayjani MA (2007) The effects of vitamin E and vitamin C supplementation on bioenergetics index. Res Sports Med 15: 249–256.

Kozan R, Ayyildiz M, Yildirim M, Agar E (2006) The effects of ethanol intake and withdrawal on penicillin-induced epileptiform activity in rats. Brain Res Bull 71: 111–115.

Kozan R, Ayyildiz M, Yildirim M, Agar E (2007) The influence of ethanol intake and its withdrawal on the anticonvulsant effect of alpha-tocopherol in the penicillin-induced epileptiform activity in rats. Neurotoxicology 28: 463–470.

Kwon TD, Lee MW, Kim KH (2014) The effect of exercise training and water extract from propolis intake on the antioxidant enzymes activity of skeletal muscle and liver in rat. J Exerc Nutrition Biochem 18: 9–17.

Mendes-da-Silva RF, Lopes-de-Morais AA, Bandim-da-Silva ME, Cavalcanti Gde A, Rodrigues AR, Andrade-da-Costa BL, Guedes RC (2014) Prooxidant versus antioxidant brain action of ascorbic acid in well-nourished and malnourished rats as a function of dose: A cortical spreading depression and malondialdehyde analysis. Neuropharmacology 86: 155–160.

Monte-Guedes CK, Alves EV, Viana-da-Silva E, Guedes RC (2011) Chronic treatment with ascorbic acid enhances cortical spreading depression in developing well-nourished and malnourished rats. Neurosci Lett 496: 191–194.

Nakken KO, Bjørholt PG, Johannessen SI, Løyning T, Lind E (1990) Effect of physical training on aerobic capacity, seizure occurrence, and serum level of antiepileptic drugs in adults with epilepsy. Epilepsia 31: 88–94.

Neeper SA, Gómez-Pinilla F, Choi J, Cotman C (1995) Exercise and brain neurotrophins. Nature 373: 109.

Nieman DC, Henson DA, McAnulty SR, McAnulty L, Swick NS, Utter AC, Vinci DM, Opiela SJ, Morrow JD (2002) Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J Appl Physiol 92: 1970–1977.

Niki E (1991) Action of ascorbic acid as a scavenger of active and stable oxygen radicals. The American Journal of Clinical Nutrition 54: 1119S–1124S.

Oliveira MS, Furian AF, Royes LFF, Fighera MR, De Carvalho J, Fiorenza NG, Mello CF (2004) Ascorbate modulates pentylenetetrazol-induced convulsions biphasically. Neuroscience 128: 721–728.

Paschalis V, Theodorou AA, Kyparos A, Dipla K, Zafeiridis A, Panayiotou G, Vrabas IS, Nikolaidis MG (2014) Low vitamin C values are linked with decreased physical performance and increased oxidative stress: reversal by vitamin C supplementation. Eur J Nutr DOI 10.1007/s00394-014-0821-x

Peake JM (2003) Vitamin C: effects of exercise and requirements with training. Int J Sport Nutr Exerc Metab 13: 125–151.

Pierce RC, Rowlett JK, Rebec GV, Bardo MT (1995) Ascorbate potentiates amphetamine-induced conditioned place preference and forebrain dopamine release in rats. Brain Research 688: 21–26.

Rosa EF, Ribeiro RF, Pereira FM, Freymüller E, Aboulafia J, Nouailhetas VL (2009) Vitamin C and E supplementation prevents mitochondrial damage of ileum myocytes caused by intense and exhaustive exercise training. J Appl Physiol 107: 1532–1538.

Sawicka-Glazer E, Czuczwar SJ (2014) Vitamin C: A new auxiliary treatment of epilepsy? Pharmacological Reports 66: 529–533.

Setkowicz Z, Mazur A (2006) Physical training decreases susceptibility to subsequent pilocarpine-induced seizures in the rat. Epilepsy Res 71: 142–148.

Souza MA, Oliveira MS, Furian AF, Rambo LM, Ribeiro LR, Lima FD, Dalla Corte LC, Silva LF, Retamoso LT, Dalla Corte CL, Puntel GO, de Avila DS, Soares FA, Fighera MR, de Mello CF, Royes LF (2009) Swimming training prevents pentylenetetrazol-induced inhibition of Na+, K+-ATPase activity, seizures, and oxidative stress. Epilepsia 50: 811–823.

Takahashi M, Hayashi S, Kakita A, Wakabayashi K, Fukuda M, Kameyama S, Tanaka R, Takahashi H, Nawa H (1999) Patients with temporal lobe epilepsy show an increase in brain-derived neurotrophic factor protein and its correlation with neuropeptide Y. Brain Res 818: 579–582.

Thompson D, Williams C, Kingsley M, Nicholas CW, Lakomy HK, McArdle F, Jackson MJ (2001) Muscle soreness and damage parameters after prolonged intermittent shuttle-running following acute vitamin C supplementation. Int J Sports Med 22: 68–75.

Tolbert LC, Thomas TN, Middaugh LD, Zemp JW (1979) Effect of ascorbic acid on neurochemical, behavioral, and physiological systems mediated by catecholamines. Life Sciences 25: 2189–2195.

Tutkun E, Ayyildiz M, Agar E (2010) Short-duration swimming exercise decreases penicillin -induced epileptiform ECoG activity in rats. Acta Neurobiol Exp (Wars) 70: 382–389.

Urso ML, Clarkson PM (2003) Oxidative stress, exercise, and antioxidant supplementation. Toxicology 189: 41–54.

Vezzani A, Ravizza T, Moneta D, Conti M, Borroni A, Rizzi M, Samanin R, Maj R (1999) Brain-derived neurotrophic factor immunoreactivity in the limbic system of rats after acute seizures and during spontaneous convulsions: temporal evolution of changes as compared to neuropeptide Y. Neuroscience 90: 1445–1461.

Vissing J, Andersen M, Diemer NH (1996) Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 16: 729–736.

Wambebe C, Sokomba E (1986) Some behavioural and EEG effects of ascorbic acid in rats. Psychopharmacology 89: 167–170.

Yamamoto N, Kabuto H, Matsumoto S, Ogawa N, Yokoi I (2002) α-Tocopheryl-L-ascorbate-2-O-phosphate diester, a hydroxyl radical scavenger, prevents the occurrence of epileptic foci in a rat model of post-traumatic epilepsy. Pathophysiology 8:205–214.

Yildirim M, Ayyildiz M, Agar E (2010) Endothelial nitric oxide synthase activity involves in the protective effect of ascorbic acid against penicillin-induced epileptiform activity. Seizure 19: 102–108.