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The protective effect of vitamin supplementation (E and E + C) on passive avoidance learning and memory during exposure to 900 MHz RFW emitted from BTS

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Abstract

Deleterious effects of exposure to electromagnetic radiation on public health have been widely studied. This study was conducted to evaluate the protective effect of vitamin supplementation (E or E + C) on passive avoidance learning (PAL) and memory in rats subjected to 900 MHz radiofrequency waves (RFW). Thirty adult male Sprague-Dawley rats (190 ± 20 g) were randomly divided into six groups as: control I (vehicle), control II (vitamin E 250 mg/kg), control III (vitamin E 100 mg/kg + L-ascorbic acid 200 mg/kg), and three exposed groups to RFW as: sham-exposed, treatment I (vitamin E), and treatment II (vitamin E + C). The duration of exposure was 30 continuous days (4 h/day). The PAL was evaluated on the last day by the shuttle box. Learning and memory of animals demonstrated as the duration of remaining within the light area, which is called the light time (LT). The sham-exposed group showed a significant decrease in LT on the learning, consolidation, and retention days compared to other groups ($p < 0.05$). Pretreatment with vitamins (E and E + C) could protect PAL against adverse effects of RFW, and the administration of vitamin E + C improved PAL performance in control III compared to control I and treatment II groups ($p < 0.05$). Administration of vitamin E + C to exposed group (treatment II) caused a significant increase in LT on the learning ($p = 0.013$), consolidation, and retention ($p = 0.009$) sessions compared to the treatment group I (vitamin E). Long-term exposure to 900 MHz RFW impaired PAL and memory, and pretreatment of vitamin (E or E + C) prevented these effects, which may be a new potential mechanism against side effects of RFW.

Keywords

Electromagnetic field, passive avoidance performance, vitamin C, vitamin E, learning and memory

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Introduction

The widespread use of cellular phones brought up the question of their possible adverse biological health hazards. Several experimental and clinical studies have implicated that exposure to mobile phone radiation leads to deleterious effects on the various body organs including reproductive system (Azimzadeh and Jelodar, 2019; Ozorak et al., 2013) and brain (Alkis et al., 2019; Bilgici et al., 2013; Ertilav et al., 2018). Use of mobile phone due to close exposure to the head has been reported to intensify headache, increase blood–brain barrier permeability, disrupt monoamine neurotransmitters, and significantly change nervous system activity (Aboul Ezz et al., 2013; Altunkaynak et al., 2016; Wang et al., 2017). Impaired learning and memory have also been widely investigated and documented following exposure to

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radiofrequency waves (RFW) (Azimzadeh et al., 2018; Saikhedkar et al., 2014; Tashiro et al., 2007).

Electromagnetic fields may affect the biological system through various mechanisms such as alteration of cell membrane potential, signal transduction, hormonal and molecular situation, while the most important of these is oxidative stress (Agarwal et al., 2011).

Both vitamin C and vitamin E have neuroprotective properties and are important redox homeostatic factors in the central nervous system (Hansen et al., 2014; Monteiro et al., 2005). The vital importance of vitamin C in the brain, and its protective and beneficial effects on learning and memory, has been reported in various experimental conditions (Beheshti et al., 2017; Jetti et al., 2016). Moreover, the administration of vitamin E has been reported to prevent memory impairment caused by sleep deprivation and oxidative stress (Aiguo et al., 2010; Alzoubi et al., 2012; Mecocci et al., 2004). Interestingly, administration of a combination of vitamins E and C was reported to improve learning and memory (Arzi et al., 2004; Hasanein and Shahidi, 2010).

We have previously demonstrated that administration of vitamin C significantly improved learning and memory in the rats exposed to 900 MHz RFW (Jelodar et al., 2018); therefore, in this study, we applied the same exposure method to examine the protective effects of vitamin supplementation (E alone and E + C in combination) on learning and memory in RFW-exposed rats.

Materials and methods

Animal experiments

Thirty adult male Sprague-Dawley rats (190 ± 20 g) were housed in polycarbonate cages in the animal room under controlled lighting (12-h light:12-h darkness) and temperature (20–22°C) conditions and had free access to pelleted food and tap water. All of the experimental procedures were carried out between 9 a.m. and 1 p.m. All investigations were conducted in accordance with the "Guiding Principles for the Care and Use of Research Animals" approved by Shiraz University (S9430259, February 2018).

Experimental design

The effect of RFW (900 MHz) on passive avoidance learning (PAL) of rats and the role of vitamin supplementation (E or E + C) were studied. Animals were randomly divided into six groups ($n = 5$) and received vitamin (E or E + C) supplementation orally (PO) once a day for 30 continuous days as follow:

Three control groups are not exposed to RFW:

Group 1: control I (received distilled water).
Group 2: control II (received vitamin E 250 mg/kg).
Group 3: control III (received a combination of vitamins E 100 mg/kg + L-ascorbic acid 200 mg/kg).

The next three groups are exposed to 900 MHz RFW (4 h/day):

Group 4: sham-exposed group (received distilled water).
Group 5: treatment I (received vitamin E 250 mg/kg).
Group 6: treatment II (received vitamins E 100 mg/kg + L-ascorbic acid 200 mg/kg).

Radiofrequency signal generator

The apparatus and procedure were essentially the same as our previous report (Azimzadeh and Jelodar, 2019). Briefly, a simulator with a 12-cm antenna emitting 900 MHz RFW circularly with the average power density of 0.6789 mW/cm^2 was used. The simulator was centrally placed 1 m away from the cages of exposed groups on the floor of the experimental room.

PAL apparatus and procedure

PAL of the animals was evaluated after 30 days of exposure to RFW by the shuttle box as previously described (Azimzadeh et al., 2018; Jelodar et al., 2018). Briefly, a two-way shuttle box (manufactured by Aryoazma Co., Tehran, Iran), which contains light and dark compartments, was used. When animals moved from the light safe compartment into the dark compartment received a foot shock of 0.6 mA (for 1 s) with a latent period of 1 s.

PAL evaluation was carried out with modifications in the method demonstrated by Bures et al. (2016). The evaluation performed in 5 days as; introduction, initial latency, learning, memory consolidation, and memory retention. Foot shock (0.6 mA, 1 s) was applied to animals in the learning day. If the animals didn't move to the dark compartment after 120 s in the course of learning, memory consolidation, and memory retention, they are considered as completely learned.

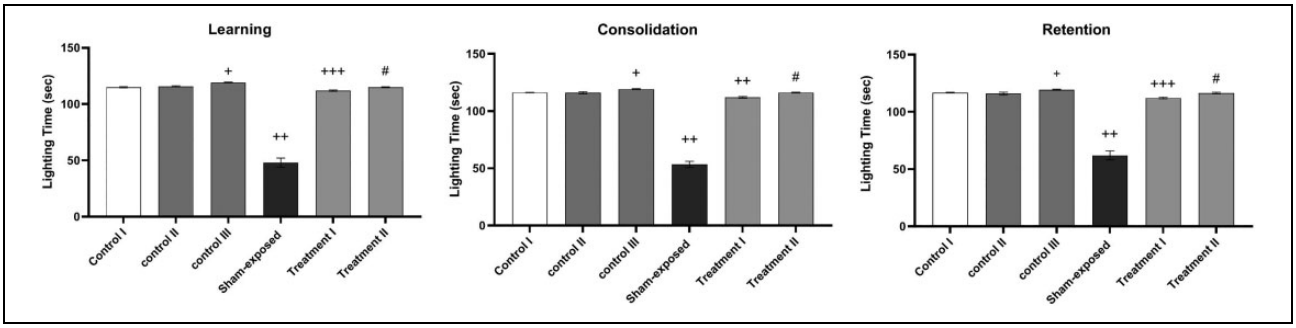


Figure 1. Protective effects of vitamin E and vitamin E + L-ascorbic acid supplementation on PAL performance (learning, consolidation, and retention sessions) in rats exposed to 900 MHz RFW (4 h/day for 30 continuous days). Columns represent mean ± SEM. Control groups (I, II, and III) are not exposed to RFW and received distilled water, vitamin E 250 mg/kg, or a combination of vitamins E (100 mg/kg) and L-ascorbic acid (200 mg/kg), respectively. Sham-exposed, treatment I and II groups are exposed to RFW and received distilled water, Vitamin E 250 mg/kg, or vitamin E 100 mg/kg + L-ascorbic acid 200 mg/kg, respectively. $^{+}$Significant difference with control I and treatment II. $^{++}$Significant difference with all groups. $^{+++}$Significant difference with control I. $^{\#}$Significant difference with treatment I ($p < 0.05$).

Statistical analysis

All data are presented as mean ± standard error and analyzed by Statistical Package for Social Sciences (SPSS, version 21.0). The nonparametric Kruskal–Wallis test was used to compare results separately for each group. Then, if results were significant, pairwise comparisons were made using the Mann–Whitney test. A value of $p < 0.05$ was considered statistically significant.

Results

Effects of vitamin (E and E + C) treatment on the PAL performance

The results of this study indicated that in the learning session (Figure 1), the sham-exposed group has a significant decrease in light time (LT) compared to all other groups (for the control I and III and treatment II, $p = 0.009$, and for control II and treatment I, $p = 0.008$). LT was significantly higher in the control I group compared to the treatment I group ($p = 0.014$). Moreover, a significant increase in LT in the control III group (received vitamin E + C) was observed compared to the control I and treatment II groups ($p = 0.008$).

Regarding memory consolidation (Figure 1) session, a significant decrease in LT was noticed in the sham-exposed group compared to all other groups (for control I, $p = 0.007$, and for control II and III and treatment I and II, $p = 0.009$). Moreover, there was a significant increase in LT in the control I group compared to the treatment I group ($p = 0.007$). Control III showed a significant increase in LT compared to control I ($p = 0.007$) and treatment II ($p = 0.008$).

On the memory retention session, the sham-exposed group showed a significant decrease in LT compared to all other groups (for control I and II and treatment I and II, $p = 0.009$, and for control III, $p = 0.008$). Also, there was a significant increase in LT in the control I group compared to the treatment I group ($p = 0.008$). Control III group also showed a significant increase in LT compared to control I ($p = 0.010$) and treatment II ($p = 0.019$) groups.

Administration of vitamin E + C to exposed group (treatment II) caused a significant increase in LT on the learning ($p = 0.013$), consolidation, and retention ($p = 0.009$) sessions compared to the treatment I group that received vitamin E alone (Figure 1).

Discussion

The present study showed that exposure to RFW (900 MHz RFW) for 30 continuous days (4 h/day) adversely affected PAL performance, which is in accordance with our previous findings (Azimzadeh et al., 2018; Jelodar et al., 2018). Similar to our findings, impaired PAL performance following exposure to RFW has been documented. In one study, exposure to 0.9 GHz/1.8 GHz (1 h/day for 4 weeks) was reported to damage the PAL and hippocampal morphology in rats (Narayanan et al., 2010). In another study, prenatal exposure to 900 MHz EMF (1 h/day, on days 13–21 of pregnancy) adversely affected female pups' learning behavior and histopathology of the hippocampus (İkinci et al., 2013). Both acute (1 week) and chronic (10 weeks) exposure to 900

MHz EMF (2 h/day) were reported to impair the passive avoidance behavior of rats, with minor effects in the chronic group (Gökçek-Saraç et al., 2017). DNA damage, demyelination of cortical neurons, astroglial activation, histopathological changes in the structures of brain such as hippocampus and other neurological or neurobehavioral disorders have been reported following exposure to 900 MHz RFW (Alkis et al., 2019; Ammari et al., 2008; Kim et al., 2017; Kivrak et al., 2017). Although the exact mechanism that is responsible for such effects of RF radiation is not well defined, the majority of studies are consensus on oxidative stress mechanism by enhancing lipid peroxidation following increase free radicals production and changing the antioxidant defense systems.

The relation between RFW and oxidative stress has widely been documented (Akbari et al., 2014; Alkis et al., 2019). In one study, rats were exposed to 900 MHz RFW (1 h/day for 3 weeks) and a significant increase in the level of oxidative stress, MDA, and advanced oxidation protein product in exposed rats was observed (Bilgici et al., 2013). Significant increases in MDA and a decrease in antioxidant parameters in the hippocampus of rats were reported following exposure to 900 MHz of mobile phone radiation (Kerman and Senol, 2012). Given that there are many similar findings in this context (Dasdag et al., 2009; Nazıroğlu and Akman, 2014), it seems that oxidative stress is the main mediator of RFW side effects.

Conversely, several reports indicated a link between oxidative stress and cognitive deficits in various experimental conditions. A study reported that impairments in passive avoidance memory induced by sleep deprivation were due to hippocampal oxidative stress (Silva et al., 2004).

Surprisingly, current results demonstrated that although supplementation with both vitamin E alone and vitamin E + C in combination prevented the adverse effects of RFW exposure on PAL performance, however, the administration of vitamin E + C to exposed group (treatment II) created significantly better results compared to the group received vitamin E alone (treatment I).

It is generally accepted that vitamin C can potentiate learning and memory processes and prevent memory deficits in various experimental conditions (Babri et al., 2015; Beheshti et al., 2017; Parle and Dhingra, 2003). Besides, the protective effects of vitamin C against RFW in different organs (Akbari et al., 2014; Jelodar et al., 2013, 2014) and PAL have already been reported (Jelodar et al., 2018).

Moreover, the protective and improving effects of treatment with vitamins C and E on cognitive function of aged mice (Arzi et al., 2004), PAL in diabetic rats (Hasanein and Shahidi, 2010), acetylcholinesterase activity and neurotransmitter levels in scopolamine-induced dementia (Lee et al., 2001) have been well documented.

Conclusion

Long-term exposure to RFW under the conditions of this study caused impairments in PAL performance. The administration of combined vitamins (E + C) showed statistically better results compared to vitamin E alone, but the results were comparable to our previous report using vitamin C alone. Although the administration of combined vitamins (E + C) gave better results in PAL compared to using vitamin C alone, the difference was not remarkable. Therefore, it seems reasonable to conclude that either antioxidant (E or C) may be effective with only marginal benefits if combined.

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