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The effect of prolong exposure to low frequency electromagnetic fields on mechanical activity of isolated aorta of male rats

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Abstract:

Background: Electromagnetic fields (EMF) have been proposed to affect cardiovascular system. This study was designed to assess the effects of long-term exposure of extremely low frequency EMF of 100 and 500 µT on vascular activity in rats.

Methods: Twenty-one Wistar adult male rats were divided equally into three groups (n=7). The first group exposed to 50 Hz, 100 µT, another group exposed to 50 Hz, 500 µT, and the third group were consider as control and were not exposed to EMF. After 210 days of exposure, The animals were anaesthetized and thoracic aorta preparation was dissected and cut into 5 mm strips were linked to force transducer that connected to AD instrument powerlab. The aortic strips inserted to organ bath chambers containing oxygenated Krebs solution (37°C). Then the aortic isometric tension was recorded after phenylephrine and acetylcholine administration.

Results: Vasorelaxation response to acetylcholine was not significantly different between three groups (p=0.60). However, the vasoconstriction response to phenylephrine in 500 µT group was less than those of other groups (p=0.035).

Conclusion: It seems that prolong exposure to LF- EMF alter vascular response to vasoactive factors via adrenergic receptor pathways.

Keyword: Low Frequency Electromagnetic Fields; Mechanical Activity; Aorta; Vascular Response; Rat

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1. Introduction

During the last decades, because of the wide-spread application of electromagnetic fields (EMFs) in everyday life, electromagnetic radiations have received high interest due to concern on the potential damaging effects on both human and animal health. Extremely low frequency electromagnetic fields (LF-EMFs) are associated with the production, transmission, and use of electricity. Several authors have previously reported an increased health risk due to exposure to EMFs at 50 and 60 Hz and it has been stated that EMFs resulted to increased tumor incidence and affected on cardiovascular system and produce oxidative stress (1-6).

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Vascular tone refers to the degree of smooth muscle contraction experienced by blood vessels. Basal Vascular tone is determined by the balanced production of vasodilator and vasoconstrictor agents such as nitric oxide (NO), prostacyclin and sympathetic nervous system. Endothelial cells as important part of the vasculature, is regarded a barrier between the blood and vessel wall which control vascular function by responding to vasoactive factors (7-9). NO induced by acetylcholine (Ach) that active muscarinic receptor localized on endothelium result in endothelium-dependent vasodilation (10, 11) Therefore NO play an important role in the regulating of basal vasodilator tone (12, 13). NO is generated by the influence of the enzyme nitric oxide synthase (NOS), which produces NO from the amino acid L-arginine. Three different isoforms of NO synthase exist in mammalian cells: endothelial NOS (eNOS or NOS III), neuronal NOS (nNOS or NOS I) and inducible NOS (iNOS or NOS II). eNOS and nNOS are expressed constitutively with Ca^{2+}-dependent manner. whereas iNOS is functionally

Ca^{2+} - independent and activated in the immune defense (14, 15). NG-nitro-L-arginine me thyl ester (L-NAME), a NO synthase inhibitor, induced blood pressure elevation suggesting that the role of NO to control of blood pressure (16-18) It is certain NO is a marker of endothelial function (19). On the other hand, the autonomic nervous system are important in considering to maintenance vascular tone. Sympathetic stimulation can influence blood pressure via mediation of postsynaptic alpha –adrenoceptors (20).

Several reports indicate that exposure to EMFs influence cardiovascular system such as blood pressure and heart rate with different pathways (21-23). Sastre et al. showed that the exposure to intermediate magnetic field reduced heart rate (24). It is certain Harte rate is mediated by sympathetic - parasympathetic balance. So that heart rate alterations may occur during exposure to EMF magnetic fields demonstrated that its effect on the autonomic nervous system (21). Some data confirm that arteriole dilation of EMF radiation is induced via Ca^{2+} concentration and NO pathway (25-27). The inhibitory effect of EMF on endotheline-1 production has been shown in experimental study (28).To summarize the related literatures in this respect, EMF may alter functions of the cardiovascular system via vasoactive factors such as NO and autonomic nervous system.

Up to now, the adverse effects of intermittent and short term exposure of LF-EMFs on the cardiovascular system have been extensively studied in both animals and humans. However, some data have been published on the effects of continued exposure to the electromagnetic radiation (Brent, 1999 and Rajaei, 2009). The importance continuous exposure of EMF on the vascular response to vasoactive factors in long term with intensities which the men are normally exposed encouraged us to investigate these effects for 7 months with intensities of 100 and 500 μT on vascular response to adrenergic and nitrergic system.

2. Method

2.1. EMFs inducing system

EMF exposure apparatus includes: solenoid as electromagnetic field generator (100 × 100 × 35 cm) with coils set parallel to each other in a wooden frame. EMF used in this study consisted of 50 Hz and 500, 100 μT intensity. The distribution of EMF flux density was measured using a gauss meter.

2.2. Animals

Twenty one wistar adult male rats (weight: 250 to 300 gr) divided in three equal groups were housed in either 500 μT magnetic field chamber (EMF500 group) or a 100 μT magnetic field chamber (EMF 100 group) or in ordinary cages in a same animal room (control group). The groups were maintained for 210 days under controlled temperature of 21 °C in 12 h light: 12 h darkness schedule with free access to food and water. The Shiraz University Ethics Committee approved the protocols and procedures. The work has been carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

2.3. Preparation of aortic strips

After seven months, animal were weighed and each rat was anaesthetized by intraperitoneal (i.p) injection of 40 mg/kg sodium pentobarbital. Thoracic aorta was dissected quickly and transferred to dish filled with ice-cold Krebs' buffer containing Kcl 0.35, $MgSO_4$ 0.29, $NaHCO_3$ 2.1, Nacl 6.89, KH_2PO_4 0.163, $CaCL_2$ 0.199 and glucose 2.17 in organ bath with pH=7.4. After removing the surrounding fat and connected tissue, it was divided in to 4-5 mm length rings (29). The ring segments were endothelium intact.

2.4. Measurement of isometric tension

In order to recorded isometric contraction, each rings was holded by tow hooks connected to a force transducer that linked to a power lab AD instrument (Panlabs, Cornella, Spain). The above noted rings was inserted in organ bath containing 20 ml of thermostated (37 °C) Krebs' buffer that was supplied to the 5% CO_2 in 95% O_2 at normal pH throughout experiment stages. Rings were equilibrated for 1 hour at an initial resting tension of 0.5 g. The Krebs solution in the bath was replaced every 15 min. Mechanical response of the ring was recorded by the following stages. 1) administration of 10-10 to 10-5 phenylephrine as the α1 agonist after 10 min cumulatively; 2) administration of 10-9 to 10-4 of acetylcholine after 10 min cumulatively (29); 3) adding of 10-4 of L-NAME as NOS inhibitor for 45 min (30); 4) administration of phenylephrine and acetylcholine in the same stage of 1 and 2. Acetylcholine was added cumulatively after the maximum contractility response phenylephrine to produce endothelium-dependent relaxation.

2.5. Statistical Analysis

Data was recorded and analyzed by SPSS software version 16. The response to phenylephrine and acetylcholine were analyzed by repeated measures ANOVA. The p value less than 0.05 was considered as significant.

3. Result

The contraction response to phenylephrine was increase dose dependently in all groups ($p<0.0001$). Significant difference in contraction response to graded phenylephrine infusion was observed in EMF rats and control ($p<0.0001$). The vasocontraction response of EMF 500 μT group to phenylephrine (as the α1 agonist) was less than those of other groups ($p=0.035$). A greater response was detected in phenylephrine response before administration L-NAME (Figure 1 and 2). The relaxation response to acetylcholine was increase dose dependently in all groups ($p<0.0001$) and don't have any difference between groups in presence or

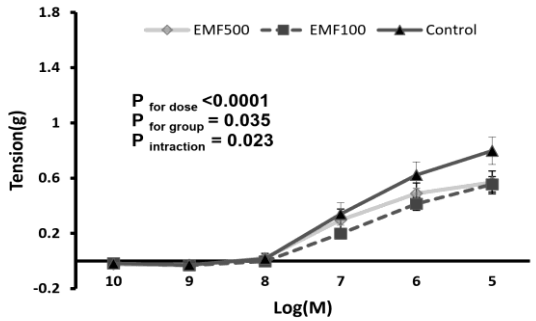

Figure 1: Contraction response to different dose of phenylephrine before NG-nitro-L-arginine methyl ester (L-NAME) administration. P value were derived from repeated measures ANOVA.

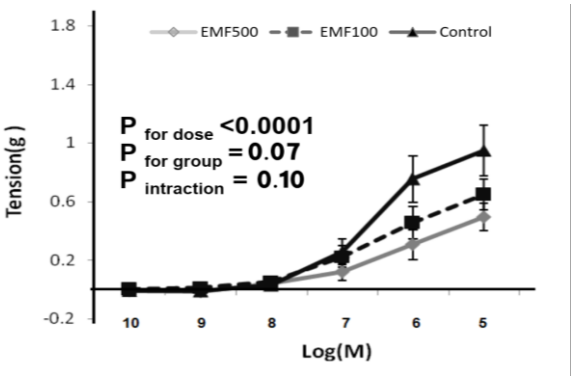

Figure 2: Contraction response to different dose of phenylephrine after NG-nitro-L-arginine methyl ester (L-NAME) administration. P value were derived from repeated measure ANOVA.

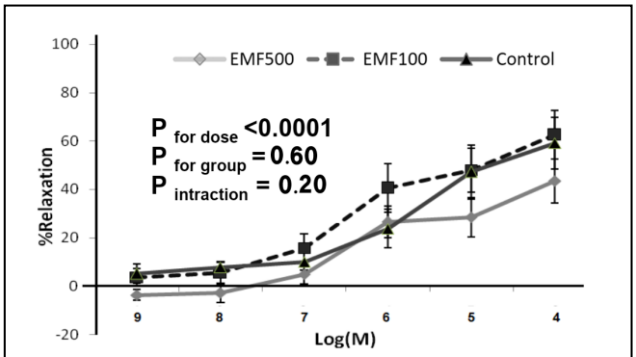

Figure 3: Relaxation response to acetylcholine infusion before NG-nitro-L-arginine methyl ester (L-NAME) administration. P value were derived from repeated measure ANOVA. Data are shown as percentage change.

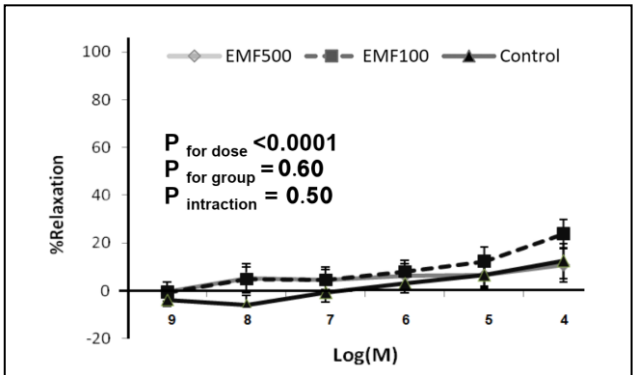

Figure 4: Relaxation response to acetylcholine infusion after NG-nitro-L-arginine methyl ester (L-NAME) administration. P value were derived from repeated measure ANOVA. Data are shown as percentage change.

absence of L-NAME (Figure 3 and 4) .The relaxation response to acetylcholine reduce after LNAME administration (Figure 4).

4. Discussion

Our major findings indicated that prolong exposure to LF-EMF decrease the vasocontraction response to phenylephrine before and after L-NAME administration but LF-EMF does not alter the relaxation response to acetylcholine compare with control group.

LF-EMF are usually found in earth environment, coming from electrical devices and appliance. Different mechanisms have been proposed to health effects because of exposure to EMFs in humans and animals (4, 22, 31). Imbalance of reactive oxygen species (ROS) and antioxidants result in oxidative stress caused by EMFs, leading to cell dysfunction and increase the risk of cardiovascular system (4, 32). It is documented that the role of EMFs on regulation of Ca^{2+} channels and Ca^{2+}-dependent cell signalling (25). Some observation demonstrated that EMF exposure could induce activity of the voltage-gated calcium channels in cell and increase intracellular Ca^{2+} (33-35). In addition, L-type voltage-gated channel blocker, verapamil inhibits EMFs effects suggesting that the role of EMF on Ca^{2+} channels (35, 36). On the other hand, the elevation of intracellular Ca^{2+} concentrations leads to change nitric oxide (NO) production (35, 37). NO as a relaxation factor diminish blood pressure (38, 39). Several studies suggest that EMFs may modulate signalling pathways, which produce NO such as Ca^{2+}-calmodulin dependent pathway (35, 37, 40). In present study was observed that inhibition of NO production with L-NAME as a nitric oxide synthase inhibitor, decrease relaxation response to acetylcholine, it provides additional support to vasodilation role of NO but EMF does not change the relaxation response to acetylcholine in presence or absence Of NO release. The field intensity of 100 and 500 μT used during 210 days duration in the present study may be not enough to induce changes in the vasorelaxation response to acetylcholine by NO signalling. It seems other pathway might be involved to EMF regulation of vascular tone. It is published that EMF exposure might modify PGE2 release (41, 42). Morimoto showed that EMF reduced endothelin-1 basal levels in endothelial cells (28).

Experimental evidence confirm that EMF can change the

production and biochemical activity of neurotransmitters such as Norepinephrine (43-45). One study suggests that The effect of Adrenaline (nonselective β-receptor agonist) was significantly suppressed by EL-EMF (46). Our findings prove that the effect of LF-EMF induce reduction the vasoconstriction response to phenylephrine before and after of L-NAME administration. Ocal and and colleagues investigated that the effect of chronic alternating current magnetic field on the contraction and relaxation mechanical response of isolated thoracic aorta rings in rats (47). Our results are in accordance with the findings of this study that showed attenuated contraction responses to phenylephrine by magnetic field.

5. Limitation

In the present study, the lack of histological and molecular evaluation to confirm the physiological findings could provide stronger evidence for the conclusion. Although attempts have been made to use the isolated aorta due to eliminate intrinsic factors, the effects of chronic exposure to EL-EMF cannot be attributed solely to the adrenergic system. Other parameters such as hormonal changes, may also changes the mechanical activity of aorta following EL-EMF. Therefore, it is suggested that biochemical, histological and cellular-molecular evaluation should be done in future studies.

6. Conclusion

It seems that prolong exposure to LF- EMF alter vascular response to vasoactive factors via adrenergic receptor pathways.

7. Acknowledgment

None.

8. Conflict of interest

The authors declare that there is no conflict of interests regarding publication of this paper

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10. Author contribution

All authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editor.

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