EFFECTS OF ELECTRIC FIELDS ON RADICAL AND ANTIOXIDANT ENZYME LEVELS

RADİKAL VE ANTİOKSİDAN ENZİM SEVİYESİNE ELEKTRİK ALANLARIN ETKİSİ

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ABSTRACT

Purpose: *Today environmental pollution by chemical substances is intensely criticized; however, electromagnetic pollution of the environment has only started to be discussed recently. Due to the development of technology and the increased use of electromagnetic energy, most of us are exposed to electric fields at different intensities, exposure periods and directions. Much research has been performed to investigate the effects of electric fields on our health and the number of these studies is increasing. The aim of this study is to determine lipid peroxidation and SOD levels in spleen and testis tissues exposed to different intensities and directions of electric fields.* ***Methods:*** *Electric potentials were applied to copper plates mounted on wooden boxes to produce electric fields with magnitudes of 1.8, 0.9 and 0.3 kV/m. Male white guinea pigs were continuously exposed to electric fields for 8 hours per day over 3 days. A total of 60 guinea pigs were exposed to electric fields. Each group of 10 guinea pigs was exposed from 9 a.m. to 5 p.m. Ten guinea pigs were used as controls. The effect of electric field exposure on malondialdehyde (MDA) and superoxide dismutase (SOD) levels was investigated for different intensities and directions.* ***Results:*** *Increasing the intensity of the electric fields caused an increase in the MDA and SOD levels detected in spleen and testis tissues.* ***Conclusion:*** *Electric fields, which we are exposed to without our being aware of it in our daily lives, may have an effect on the radical synthesis in our bodies.*

Key Words: *Electric Field, Radicals, Antioxidant Enzymes, Malondialdehyde, Superoxide Dismutase.*

ÖZET

Amaç: *Günümüzde çevrenin kimyasal maddelerle kirletilmesi yoğun eleştirilere neden olurken; elektromagnetik çevre kirlenmesi gündemimize yeni girmeye başlamış konulardandır. Teknolojinin gelişimi ile elektromagnetik enerjinin kullanımının giderek yaygınlaşması nedeniyle çoğumuz sürekli olarak değişik şiddet, süre ve doğrultularda elektrik alanlara daha çok maruz kalmaktayız. Elektrik alanların sağlığımız üzerindeki etkilerini araştırmak amacıyla çok sayıda çalışma yapılmakta ve bu araştırmalar gün geçtikce artmaktadır. Bu çalışmada farklı şiddet ve doğrultularda uygulanan elektrik alanların dalak ve testis dokularında lipid peroksidasyon ve antioksidan enzimler üzerindeki etkileri kobaylarda incelendi.* ***Metod:*** *Tahta kafesler üzerine monte edilmiş olan bakır plakalara potansiyel fark uygulanarak 1.8, 0.9 ve 0.3 kV/m şiddetinde elektrik alanlar oluşturuldu. Her grupta 10 adet kobay olmak üzere toplam 60 adet erkek beyaz kobay günde 8 saat olmak üzere 3 gün boyunca elektrik alanlara sürekli olarak maruz bırakıldı. Elektrik alan uygulama işlemine saat 9.00'da başlanıp 17.00'de son verildi. On adet kobay ise kontrol grubunu oluşturdu. Farklı şiddet ve doğrultularda uygulanan elektrik alanların malondialdehit (MDA) ve superoksit dismutaz (SOD) seviyelerine etkisi araştırıldı.* ***Sonuç:*** *Dalak ve testis dokularında, uygulanan elektrik alanın şiddeti arttıkca MDA ve SOD seviyelerinde artış tespit edilmiştir.* ***Tartışma:*** *Günlük yaşamda farkına varmadan maruz kaldığımız şiddet ve doğrultulardaki elektrik alanlardan vücudumuzdaki radikal sentezinin etkilenebileceğini söyleyebiliriz.*

Anahtar Kelimeler: *Elektrik Alan, Radikaller, Antioksidan Enzimler, Malondialdehit, Süperoksit Dismutaz.*

INTRODUCTION

All living organisms are continuously exposed to both natural and man-made electric fields. Living organisms interact with this environment of electric and magnetic fields. The question is whether they have adapted to this environment of electric and magnetic fields. We think that they have not yet accomplished the process of adaptation.

There has been renewed concern in recent years about the biological effects of electric (E) fields. Public concern has increased about the possible health risks of exposure to electromagnetic fields (EMFs) generated by electric power distribution systems. Much of this concern has focused on the relationship between chronic exposure and cancer. Numerous epidemiological reports suggest a link between occupational or residential exposure to EMFs and increased cancer risk. There is accumulating evidence from epidemiological studies that exposure to EMFs may increase the incidence of various types of cancer, particularly leukemia, brain tumors, and breast cancer (1-3). However, there is little understanding of the nature of the interaction between EMFs and biological systems. The observed effects include changes in enzyme activity and radical synthesis.

Free radicals, such as superoxide anions ($O2^{\cdot -}$), which are generated by an electrical stimulus, show high chemical reactivity and as a result have a relatively short lifetime in the free state (4-6). The participation of free radical biochemistry in several pathologies and aging has been demonstrated (7). The free radical oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation and the detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free-radical reactions (8). The increase in radicals can be traced to the variation in malondialdehyde (MDA) quantities, which is an end product of lipid peroxidation (9).

The importance of the enzyme superoxide dismutase (SOD) in eliminating these radicals is well established (4,10,11). SOD scavenges the superoxide radicals by catalyzing the reactive $O2^{\cdot -}$ species into dioxygen and oxygen peroxide, thereby protecting cells against the reactive oxygen species produced by the electric field or other mechanisms (12,13).

The aim of this study was to examine radical synthesis in spleen and testis tissues under the effect of exogenous electric fields applied in different intensitities and directions.

MATERIALS AND METHODS

Electric Field Exposure: Guinea pigs were housed in wooden cages with dimensions of 50 cm x 50 cm x 14 cm and were exposed to electric fields. For vertical field exposure, copper plates were mounted on the top and bottom faces of the cages to form the parallel plates of a capacitor. The copper plate spacing was 14 cm and the dimensions of the plates were 50 cm x 50 cm x 0.1 cm.

The positive probe of the DC power supply was always connected to the upper plate and the negative probe to the lower plate. For horizontal field exposure, the copper plates were mounted on the left and right faces of the cages. The positive probe of the power supply was always connected to the left plate and the negative probe to the right plate. The potential differences were controlled continuously throughout the experiment and were kept constant with the aid of a multi-meter connected to the circuit.

Electric potentials were applied to the copper plates mounted on the wooden boxes to produce electric fields with magnitudes of 0.3, 0.9 and 1.8 kV/m. Male white guinea pigs (150-200 g) were continuously exposed to both horizontal and vertical electric fields for 8 hours per day over 3 days. A total of 60 guinea pigs were exposed to electric fields. The animals were divided into 6 separate groups each with 10 guinea pigs and each group was subjected to various combinations of electric field intensities and directions. Ten guinea pigs were used as controls and were kept under the same conditions without being exposed to any electric field. The animals were housed in cages for 3 days.

Taking into consideration the fact that placing more than one animal per cage would create a stress factor, only one animal was placed per cage during each electric field exposure period. All the animals were kept at a room temperature of 23 °C, a day and night cycle of 12 hours and a relative humidity of 50% and fed ad libitum a standard lab chow and carrot.

Spleen and testis tissue samples were rapidly removed after decapitation.

Measurement of Tissue Malondialdehyde Level: Tissue samples were weighed and homogenized in cold 1.15% KCl to make 10% homogenate. Then 0.5 ml of the 10% homogenate was pipetted into a 10 ml centrifuging tube to which 3 ml of 1% phosphoric acid and 1 ml 0.6% TBA aqueous solution were added. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol was added, followed by vigorous mixing. The butanol phase was separated by centrifugation and absorbance was measured at 535 and 520 nm. The difference was used as the TBA value. The results were expressed in nanomoles per gram of tissue (nmol/g tissue) (14).

Measurement of Tissue Superoxide Dismutase Level: Total SOD activities were measured by the nitroblue tetrazolium inhibition assay (15). The protein concentration was measured by the method described by Lowry et al. (16). The results were expressed in units per liter (U/mg protein).

Statistical Analysis: For each group, MDA and SOD contents of tissues from groups exposed to an electric field and their controls were compared by Mann-Whitney U test. The results and statistical significance are given in Tables 1-2, Fig. 1-4.

RESULTS AND DISCUSSION

Electric fields affect the selective transport of ions or molecules through the membrane. They change the build-up of layers at the surface and change the way new molecules are incorporated into the membrane or are bound to its surface. The result of changes in the transport of molecules or ions across cell membranes is changes in the performance of the cells and, in turn, of the organs of which they are a part (13,17-19).

Cellular organization and function can be described in terms of structural and regulatory interactions (3). On a molecular level interactions between proteins, lipids, carbohydrates and other compounds can be described in terms of EM field effects between participating molecules (20). Although the characteristics of the associated EM fields may be complex (particularly for large molecules, such as proteins), biological reactions are essentially electromagnetic. From this perspective, it is reasonable to consider that biological reactions may be influenced by EM fields.

In this investigation, statistically significant differences were found between the MDA and SOD contents of the spleen and kidney of the groups exposed to electric fields and of those of the control groups (Tables 1-2, Fig. 1-2). Increasing the intensity of the electric fields resulted in an increase in the MDA and SOD levels detected in all tissues. For electric field exposure, statistically significant elevations in MDA and SOD levels were found starting at 0.9 kV/m for the spleen and testis tissues.

Table-1: Spleen and testis tissue MDA levels (nmol/g tissue) of electric field exposed and control groups. The values represent the least squares means ± standard deviation (X ± SD).

Electric Field	Spleen	Testis
1.8 kV/m Vertical E	0.195±0.023**	0.086±0.014**
1.8 kV/m Horizontal E	0.192±0.021**	0.082±0.010**
0.9 kV/m Vertical E	0.114±0.015*	0.046±0.006*
0.9 kV/m Horizontal E	0.111±0.013*	0.044±0.005*
0.3 kV/m Vertical E	0.095±0.011	0.029±0.004
0.3 kV/m Horizontal E	0.093±0.010	0.028±0.004
Control	0.088±0.009	0.024±0.003

* $p < 0.05$, ** $p < 0.01$

Table-2: Spleen and testis SOD levels (U/mg protein) of electric field exposed and control groups. The values represent the least squares means ± standard deviation (X ± SD).

Electric Field	Spleen	Testis
1.8 kV/m Vertical E	14.43±1.33**	12.98±1.05**
1.8 kV/m Horizontal E	14.40±1.30**	12.94±1.02**
0.9 kV/m Vertical E	12.18±0.989*	8.48±0.58*
0.9 kV/m Horizontal E	12.15±0.988*	8.46±0.78*
0.3 kV/m Vertical E	9.06±0.714	6.12±0.64
0.3 kV/m Horizontal E	9.04±0.712	6.09±0.61
Control	8.35±0.701	6.00±0.58

*: $p < 0.05$, **: $p < 0.01$

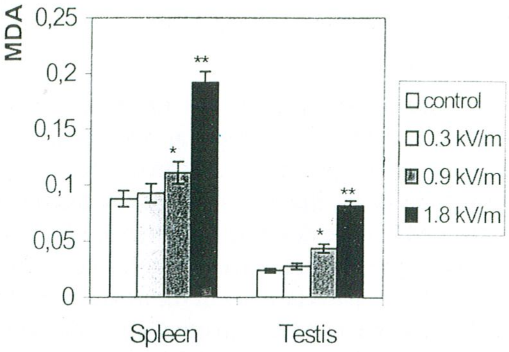

Fig. 1: MDA level of spleen and testis tissues (nmol/g tissue) under vertical E field exposure p<0.05, ** p<0.01

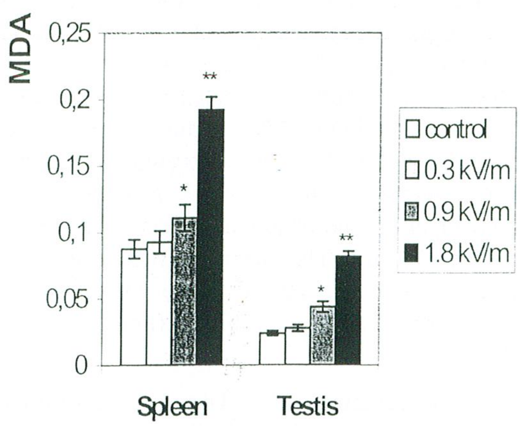

Fig. 2: MDA level of spleen and testis tissues (nmol/g tissue) under horizontal E field exposure * p<0.05, ** p<0.01

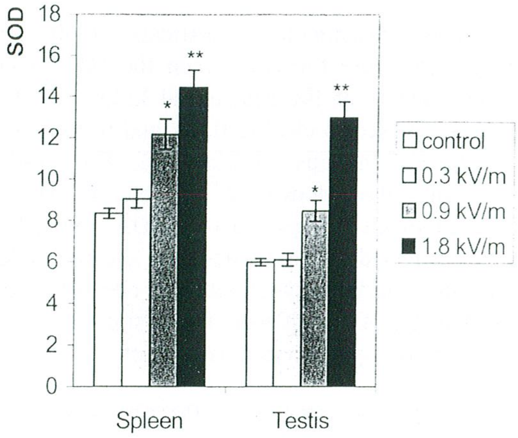

Fig. 3: SOD level of spleen and testis tissues (U/mg protein) under vertical E field exposure *p < 0.05, ** p < 0.01

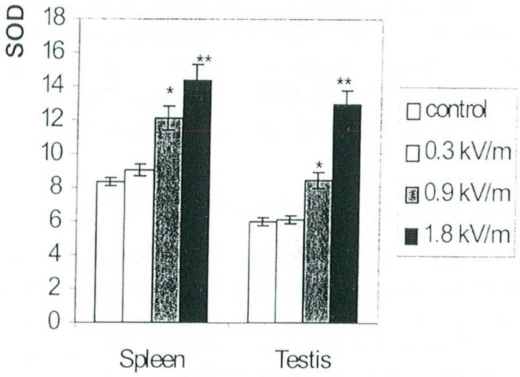

Fig. 4: SOD level of spleen and testis tissues (U/mg protein) under horizontal E field exposure * p < 0.05, ** p < 0.01

In a previous study we observed that increasing the electric field strength results in an increase in the hydroxyproline content of liver, lung and kidney tissues (18,21). Benov also showed that when the intensity of the electric field was increased, the MDA level increased proportionally (13). In this investigation, both the vertical and horizontal application of 1.8 and 0.9 kV/m electric fields increased SOD and MDA levels significantly more than the application of 0.3 kV/m (Fig. 1-2).

Another important parameter that has to be taken into consideration during electric field applications is the direction of the field applied. In this study, the vertical and horizontal application of electric fields increased MDA and SOD levels compared to the controls (Fig. 1-2). In an earlier investigation into the effects of vertical and horizontal electric field exposure on the protein synthesis of liver, kidney and lung tissues, we observed that the vertical electric field exposure had a greater effect than the horizontal exposure (18,21). Marino also determined that vertical electric field exposure had a larger effect than horizontal exposure (22). In research performed in plant roots, it was also noted that the growth speed of roots is affected more by vertical electric field exposure (23-25). In this study, the vertical application of electric fields was also found to have a greater effect than the horizontal one, but the differences were not statistically significant ($p>0.05$).

In this investigation, exposure to electric fields resulted in an increase in MDA levels in the spleen and testis tissues of guinea pigs. We hypothesize that, as a result of energy transfer from the electric field to the applied tissue, molecular O2 was transformed to free radicals and, as a result of this increase in the $O_2^{\cdot -}$ radicals, MDA levels in those tissues increased (18).

In both vertical and horizontal E field exposures, there was an increase in SOD levels along with that in MDA levels. The mechanisms associated with this are unclear. Enhanced production of superoxide radicals might increase synthesis of SOD. A similar result was observed by Yeh et al. (26). The electric fields, which we are exposed to without our being aware of it in our daily lives, may have an effect on the radical synthesis in our bodies.

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