

Endometrial Apoptosis Induced by a 900-MHz Mobile Phone: Preventive Effects of Vitamins E and C

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ABSTRACT

Numerous reports have described the effects induced by an electromagnetic field (EMF) in various cellular systems. The purposes of this study were to examine oxidative stress that promotes production of reactive oxygen species induced by a 900-megahertz (MHz) mobile phone and the possible ameliorating effects of vitamins E and C on endometrial tissue against EMF-induced endometrial impairment and apoptosis in rats. Animals were randomly grouped as follows: (1) sham-operated control group (n=8), (2) 900 MHz EMF-exposed group (n=8; 30 min/d for 30 d), and (3) 900 MHz EMF-exposed group, treated with vitamins E and C (n=8; 50 mg/kg intramuscularly and 20 mg/kg body weight intraperitoneally before daily EMF exposure). Malondialdehyde (an index of lipid peroxidation) was used as a marker of oxidative stress-induced endometrial impairment; Bcl-2, Bax, caspase-3, and caspase-8 were assessed

immunohistochemically. In this study, increased malondialdehyde levels in endometrial tissue and apoptosis illustrated the role of the oxidative mechanism induced by exposure to a 900-MHz mobile phone-like device and vitamins E and C; via free radical scavenging and antioxidant properties, oxidative tissue injury and apoptosis were ameliorated in rat endometrium. In conclusion, exposure to 900-MHz radiation emitted by mobile phones may cause endometrial apoptosis and oxidative stress, but treatment with vitamins E and C can diminish these changes and may have a beneficial effect in preventing endometrial changes in rats.

Keywords: | apoptosis; endometrium; mobile phone; oxidative stress;
| vitamin C; vitamin E

INTRODUCTION

The biologic effects of electromagnetic fields (EMFs) and their consequences for human health are the subjects of scientific interest and the focus of great public debate. There is controversy regarding epidemiologic studies that have reported a relationship between magnetic field exposure and human disease.¹ Mobile phones that emit an EMF of 900 megahertz (MHz) are commonly used in many countries. Although many animal studies have attempted to evaluate the effects of exposure to an EMF on reproduction, the results have been contradictory. Many studies have concluded that exposure to an EMF can have adverse effects on reproduction and fetal development; a relationship between long-term exposure to an EMF and fetal loss has been suggested.²⁻⁴ Other studies have reported no adverse effects on reproduction or fertility in mice and humans exposed to an EMF.⁵⁻⁷

Some studies have shown that oxidative stress may be an important component in the mechanism of damage caused by EMFs.⁸⁻¹⁰ Oxidative stress affects a variety of cell functions. EMFs may induce oxidative stress that leads to the generation of free radicals and alterations in antioxidants or reactive oxygen species (ROS)-scavenging enzymes.^{11,12} EMFs are also capable of inducing programmed cell death (apoptosis) through multifunctional pathways. Numerous studies have examined the apoptotic process in magnetic field-exposed cells.¹³ However, because magnetic field-induced apoptosis is poorly understood, great heterogeneity in cell type, magnetic field characteristics (eg, static, dynamic, frequency, intensity, pulsed, continuous), exposure time, methods of detecting and evaluating apoptosis, and so forth, were noted in these studies.¹³

Among numerous factors that may affect apoptosis, caspases, a family of cysteine proteases, are widely recognized to play fundamental roles in apoptosis; in addition, the active site cysteine residues of caspases are highly sensitive to oxidation. Caspases are a family of cysteine proteases that are present in cytosol as inactive proenzymes, but they are activated when apoptosis is initiated, playing an essential role at various stages. Caspase-3, one of the key executioners of apoptosis, is responsible, either partially or totally, for the proteolytic cleavage of many structural and regulatory proteins.¹⁴ It is generally accepted that the ratio of proapoptotic to antiapoptotic Bcl-2 family proteins is the critical determinant in the fate of the cell, with an excess of Bcl-2 resulting in cell survival and an excess of Bax causing cell death.¹⁵ Thus, apoptotic regulator proteins seem to play a major role in the regulation of cell death.

Recently, several studies have proposed a role for oxidative stress in the induction of apoptosis, and it has been observed that various antioxidants, such as N-acetylcysteine and melatonin, can inhibit cell death.¹⁶ Nonenzymatic antioxidants such as vitamins E and C may act to overcome oxidative stress because they are part of the antioxidant system. Antioxidants play an important role in preventing free radical-mediated damage by directly scavenging free radicals. α -Tocopherol is one of the most widely distributed naturally occurring and biologically active antioxidants in the biologic system. It protects against lipid peroxidation (LPO) most efficiently through its chain-breaking antioxidant action. Intracellularly, it is associated with lipid-rich membranes such as mitochondria and microsomes. In contrast to α -tocopherol, ascorbic acid is hydrophilic and functions better in the aqueous environment than does α -tocopherol. Moreover, it can restore the antioxidant properties of oxidized tocopherol, suggesting that a major function of ascorbic acid is to recycle the tocopheroxyl radical. Some studies have reported that a combination of vitamins E and C reduces LPO and apoptosis caused by toxic substances.^{17,18}

Previous studies have emphasized the *in vivo* effects of short- and long-term EMF exposure on LPO and antioxidant enzymes.¹⁹⁻²³ However, investigators in the present study could find no studies in which the peroxidative effects of an EMF on endometrial apoptosis were explored. Therefore, the present study had the following objectives: (1) to investigate the effects of an EMF on endometrium through immunohistochemical observations of apoptosis and LPO in the rat; and (2) to explore the ameliorating effects of a combination of vitamins E and C against EMF damage in the rat.

MATERIALS AND METHODS

Animal Model

Twenty-four 16-wk-old, sexually mature female Wistar albino rats that weighed 170 to 200 g and had had at least 3 consecutive regular estrous cycles, were used in this study. The rats were kept in the laboratory for at least 3 wk before experiments began. Before the start of the study, all procedures were approved by the Animal Care Laboratory of Suleyman Demirel University Faculty of Medicine Institutional Review Board. Rats were maintained on a 12-h light/12-h dark cycle in a temperature-regulated ($23\pm1^{\circ}\text{C}$) animal room with water and food always available. Daily phases of the estrous cycle and body weight were recorded throughout the experiment. Before EMF exposure, animals were anesthetized with pentobarbital Na (40–60 mg/kg).

Experimental Design

Rats used in this study were randomly grouped (8 each) as follows: (1) Sham-operated control animals were injected with isotonic saline solution. These animals were also placed in a tube with the same environmental room conditions as the exposure groups, but without exposure to an EMF (exposure device off group, group I). (2) Rats were exposed to a 900-MHz EMF (EMF group, group II). (3) Rats were exposed to a 900-MHz EMF and were given vitamin treatment (EMF+Vit group, group III). Study groups (groups II and III) were exposed to a 900-MHz EMF for 30 min/d over 30 d. Vitamin E, as α -tocopherol acetate (Evigen; Aksu Farma,

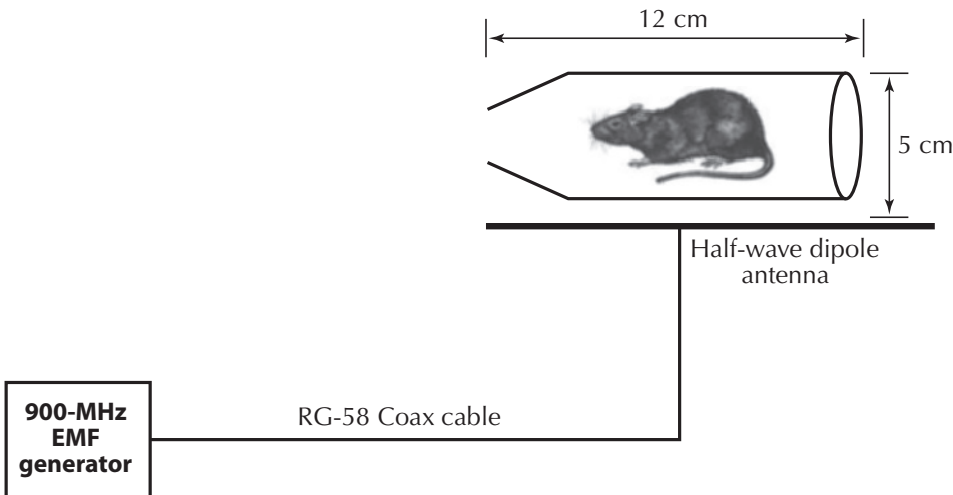
Istanbul, Turkey), and vitamin C, as sodium L-ascorbate (Redoxon; Roche, Basel, Switzerland), were injected intramuscularly at a dose of 50 mg/kg body weight^{24,25} and intraperitoneally for a dose of 20 mg/kg body weight,^{26,27} respectively, before EMF exposure. All other groups of rats (groups I and II) received the same amount of physiologic saline on a daily basis.

At the end of the study, rats were killed while in the estrous phase, and the endometrium was dissected and homogenized with phosphate buffer (pH 7.4). Unbroken cells, cell debris, and nuclei underwent sedimentation at $2000 \times g$ for 10 min, and the supernatant was pipetted into plastic tubes and stored at -70°C until it was assayed.

Exposure Device

A special exposure device with exposure antennae for insulated exposure that emitted a 900-MHz pulse-modulated (217 Hertz [Hz] repetition cycle) EMF (2 W peak output power and $1.04 \text{ mW}/\text{cm}^2$ power density) was used in the study. The specific energy absorption rate ranged between 0.016 and $4 \text{ W}/\text{kg}$ (for the whole body). Power density and specific energy absorption rate were measured at the Electromagnetic Compatibility Laboratory of the Department of Electronics and Communication Engineering (Suleyman Demirel University, Isparta, Turkey). The exposure system for an animal consisted of a plastic tube cage (length, 12 cm; diameter, 5 cm) and a dipole antenna (Fig 1). The whole body of the rat was positioned close to the dipole antenna, and the tube was ventilated from head to tail to decrease stress on the rat while in the tube.

Fig 1. A schema of a 900-MHz EMF exposure device setup (± 1 of the antennas of the device is shown).



Estrous Cycle

The daily phases of the estrous cycle and body weight were recorded throughout the experiment. After the final treatment days, control and treated animals awaited the onset of the estrous phase. The phases of the estrous cycle were determined through observation of a vaginal smear in the morning (8:00 to 10:00 AM), as described by Zarrow et al.²⁸

Biochemical Analysis

Malondialdehyde (MDA) levels (an indicator of free radical generation that increases at the end of the LPO) were estimated by the double-heating method of Draper and Hadley.²⁹ This method requires spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) combined with MDA. For this purpose, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL supernatant in each centrifuge tube, and tubes were placed in a boiling water bath for 15 min. After they had cooled in tap water, the tubes were centrifuged at 1000 g for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L TBA solution in a test tube. The tube was placed in a boiling water bath for 15 min. The solution was then cooled in tap water, and its absorbance was measured with a spectrophotometer (Shimadzu UV-1601; Shimadzu Corporation, Kyoto, Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA/TBA complex (absorbance coefficient = 1.56×10^5 cm/M) and is expressed as nanomoles per gram of wet tissue.

Immunohistochemical Methods

Tissue samples from each group were fixed in neutral formalin for 72 h and processed for paraffin embedding. Sections of 4- to 5- μ m thickness were processed for polylysine microscope slides. For immunohistochemical examination, slides were stored in a microwave oven in 0.01 M citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was blocked by 20 min of incubation with 0.3% hydrogen peroxidase. Slides were tested with mouse monoclonal anti-caspase-3 antibody (rabbit polyclonal antibody Ab-4, 1 mg/mL; NewMarker, Fremont, Calif, USA), caspase-8 (rabbit polyclonal antibody Ab-4, 1 mg/mL; NewMarker), Bax (rabbit polyclonal antibody Ab-1, 3525; NewMarker), and Bcl-2 (rabbit polyclonal antibody Ab-1, 6837; NewMarker). Sections were tested with the streptavidin-biotin peroxidase kit (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP; LabVision, Fremont, Calif, USA), and after incubation, the reaction product was detected with diaminobenzidine. Finally, sections were counterstained with Mayer's hematoxylin, mounted with mounting medium, and examined with the Olympus BH2 photo-light microscope (Olympus America, Inc., Center Valley, Pa, USA). Negative control was attained during the primary antibody application stage.

Two independent observers who were blind to the treatment selection calculated the immunolabeling score evaluations twice. Labeling intensity was graded semiquantitatively, and the HSCORE (index) was calculated with the following equation: $HSCORE = \sum P_i(i+1)$, where i = intensity of labeling with a value of 1, 2, or 3 (weak, moderate, or strong, respectively), and P_i = percentage of labeled epithelial and stromal cells for each intensity, ranging from 0% to 100%. Statistical differences were calculated with the Mann-Whitney U test. Results were expressed as mean $\pm$ SEM (standard error of the mean). $P < .05$ was considered to be statistically significant.

Statistical Analysis

Data were presented as means $\pm$ SD (standard deviation). The Statistical Package for the Social Sciences (SPSS), version 12.0 (SPSS Inc., Chicago, Ill, USA), was used for statistical analysis. A 1-way analysis of variance was applied to data so that significant differences could be detected initially. At the second step, Tukey's post hoc test was used to compare groups. Differences were considered significant at $P<.05$.

RESULTS

Uterine and Body Weight

No significant changes were noted in the mean weights of the uterus and the body between control, group II, and group III. No deaths were reported in any of the groups.

Estrous Cycle

Physiologic saline given to control rats showed normal estrous cycles and normal duration of each phase of the estrous cycle over the 4-wk treatment period. The EMF group (group II) and the EMF group that received vitamins E and C (group III) showed no significant changes in cycle length.

Biochemical Findings

Endometrial tissue levels of MDA in the EMF-exposed group (group II) were higher ($P<.01$) than those in the control group (group I). Vitamin E plus vitamin C administration in group III significantly reduced MDA levels ($P<.01$). A statistically significant difference in MDA levels was also observed between group III and the control group (group I) ($P<.01$) (Fig 2).

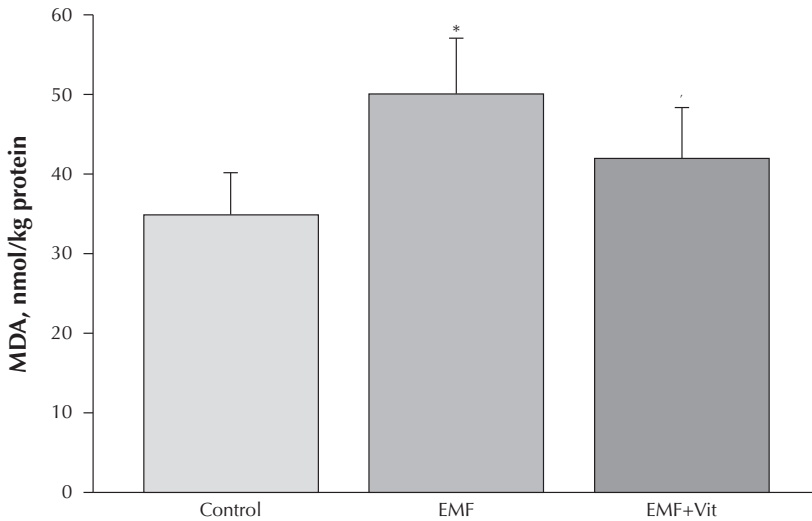
Immunohistochemical Observation

All rats were killed during the estrous cycle.

Caspase-3 Reactivity

In the control group, very weak apical cytoplasmic reactivity was seen in the endometrial surface epithelium. Weak membranous staining was noted in the endothelial cells of stromal capillaries. No caspase-3 reactivity was reported in stromal glandular cells (Fig 3A). In the EMF-applied group, mild to moderate cytoplasmic reactivity was detected. In stromal cells and capillary endothelial cells of stroma, moderate to strong immunostaining was observed (Fig 3B). In the EMF+Vit-treated group, caspase-3 immunostaining was not detected in endometrial sections (Fig 3C). Immunolabeling intensities of caspase-3 in epithelial and stromal cells of the endometrium significantly increased in the EMF group compared with the control group ($P<.01$). Immunolabeling intensities of caspase-3 in epithelial and stromal cells of the endometrium significantly decreased in the EMF+Vit group compared with the EMF group ($P<.01$). The results are shown in the Table.

Fig 2. Levels of MDA in endometrium homogenates of rats in the control, EMF exposed, and EMF+Vit groups.



* $P<.01$ =EMF vs control.

* $P<.01$ =EMF+Vit vs control.

* $P<.01$ =EMF+Vit vs EMF.

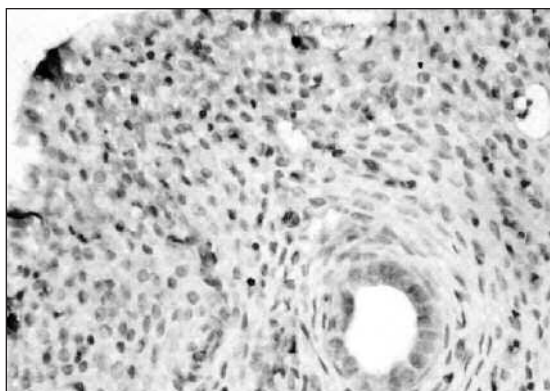
Caspase-8 Immunoreactivity

In the control group, very weak apical cytoplasmic reactivity was seen in the endometrial surface epithelial cells. In endothelial cells of stromal capillaries, weak membranous staining was noted. No caspase-8 reactivity was evident in glandular cells and stromal cells (Fig 4A). In the EMF-exposed group, moderate cytoplasmic reactivity was seen in endometrial surface epithelial cells. However, strong staining was detected in stromal cells and capillary endothelial cells (Fig 4B). In the EMF+Vit-treated group, very weak cytoplasmic staining was observed in the endometrial epithelium compared with the previous group (Fig 4C). Statistical differences in immunolabeling intensities of caspase-8 were reported among the EMF group, the EMF+Vit group, and the control group ($P<.01$). The results are shown in the Table.

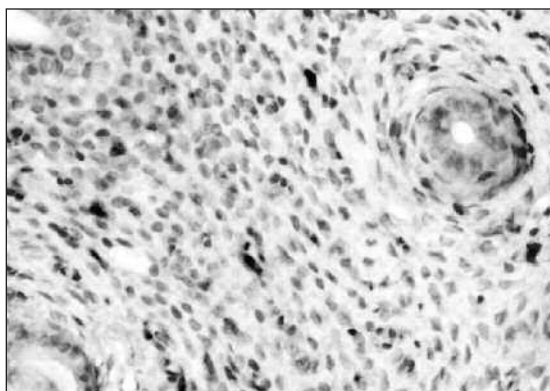
Intense immunolabeling of Bax was detected in surface epithelial cells, glandular epithelial cells, and stromal cells of the endometrium in the EMF group (Fig 5A); this was significantly greater than the immunolabeling observed in samples from controls and the EMF+Vit group ($P<.01$) (Figs 5B, 5C). In contrast, weak immunolabeling of Bcl-2 was seen in epithelial, glandular, and stromal cells in the EMF group compared with the control group ($P<.01$) (Figs 6A, 6B, 6C). These differences were found to be statistically significant ($P<.01$; Table). The ratio of Bcl-2 to Bax immunoreactivity was therefore different in the 2 groups, with detectable increases in apoptosis markers in the EMF group.

Fig 3. Immunolabeling of caspase-3 activity.

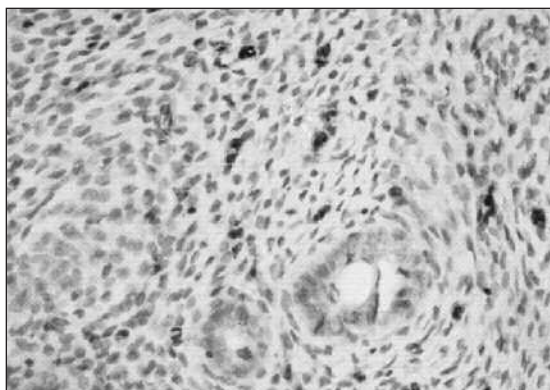
A



B



C



Weak staining in the endothelial cells of stromal capillaries in the uterine epithelium of the control group and negative staining in the stromal glandular cells for caspase-3 were detected (**A**). Moderate to strong immunostaining was detected in stromal cells and endothelial cells of stroma of the EMF group (**B**). Negative immunostaining was detected in stromal components of the EMF+Vit-treated group (**C**).

Immunolabeling Intensities of Caspase-3, Caspase-8, Bcl-2, and Bax in Epithelial and Stromal Cells of the Endometrium of the Control, EMF+Vit, and EMF Groups

	Caspase-3	Caspase-8	Bcl-2	Bax
Control group				
Epithelial cells	34.8±1.96*	28.4±2.03*	8.2±1.26*	24.4±5.14*
Stromal cells	31.5±1.3†	25.2±0.6†	6.6±1.02†	22.1±4.28†
EMF+Vit group				
Epithelial cells	37.4±2.83‡	30.5±1.04‡	7.4±1.18‡	26.2±3.96‡
Stromal cells	33.7±1.55§	27.3±0.5§	6.2±1.04§	23.4±4.82§
EMF group				
Epithelial cells	48.7±1.72*†	39.1±1.01*‡	5.2±1.56*‡	36.4±6.86*‡
Stromal cells	45.2±1.96†§	32.5±2.09†§	4.8±1.44†§	32.3±5.48†§

**P*<.01 control vs EMF groups.

†*P*<.01 EMF+Vit vs EMF groups.

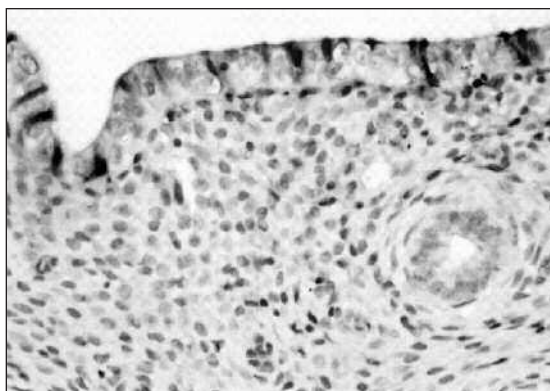
‡*P*<.01 control vs EMF groups.

§*P*<.01 EMF+Vit vs EMF groups.

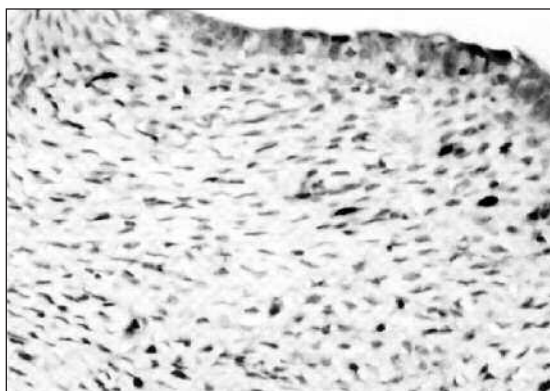
HSCORE values are expressed as means±SEM.

Fig 4. Immunolabeling of caspase-8 activity.

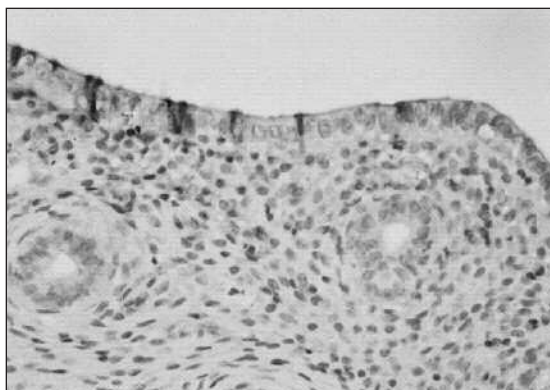
A



B



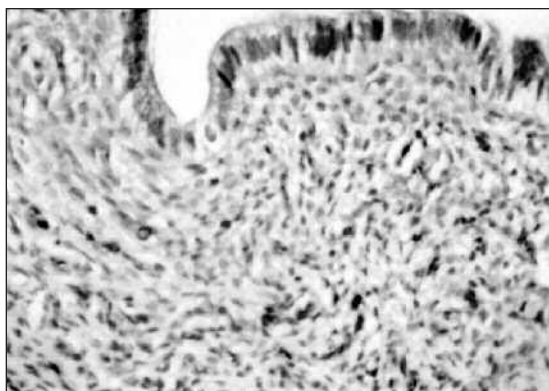
C



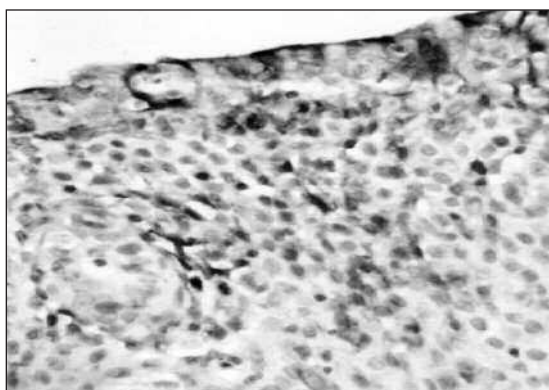
Very weak apical cytoplasmic reactivity was detected in surface epithelial cells, whereas negative immunostaining was present in glandular cells and stromal cells in the control group (A). Strong immunostaining was detected in stromal components, whereas moderate cytoplasmic reactivity was detected in surface epithelial cells in the EMF group (B). Very weak cytoplasmic staining in the endometrial epithelial cells was detected in the EMF+Vit-treated group (C).

Fig 5. Immunolabeling of Bax activity.

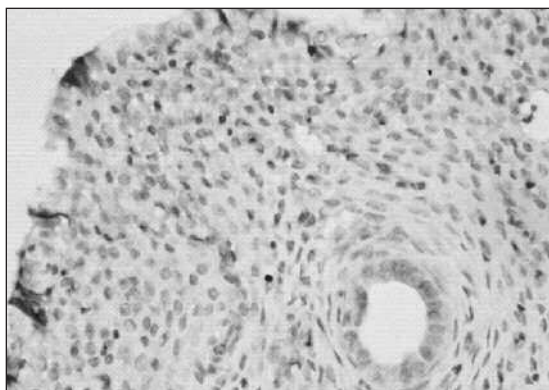
A



B



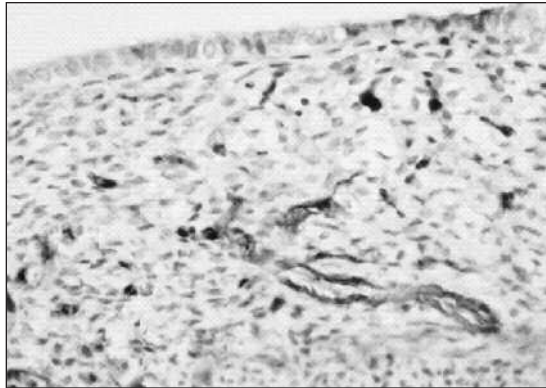
C



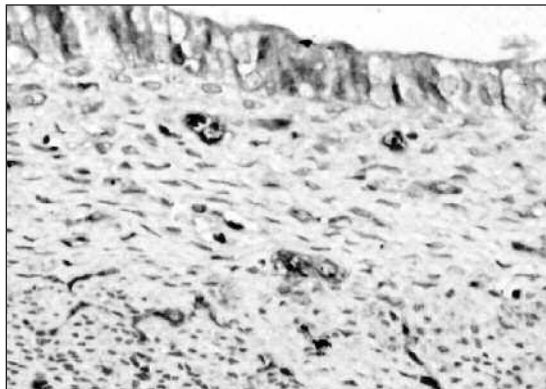
Intense immunolabeling of Bax was detected in surface epithelial cells, glandular epithelial cells, and stromal cells of the endometrium in the EMF group (A). This was significantly greater than the immunolabeling observed in samples from controls and the EMF+Vit-treated group (B, C).

Fig 6. Immunolabeling of Bcl-2 activity.

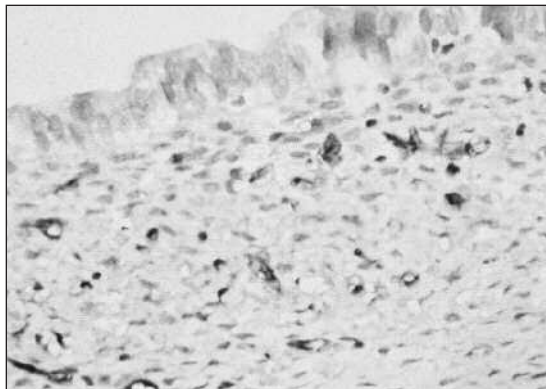
A



B



C



Weak immunolabeling of Bcl-2 was seen in epithelial, glandular, and stromal cells in the EMF group compared with the control group (A, B, C).

DISCUSSION

The use of mobile phones is one of the fastest growing technological developments in modern society. In many countries, 900-MHz EMF-emitting mobile phones are commonly used. The close proximity of the antenna of such a device to the abdominal organs has raised concerns about biologic interactions between EMF and the uterine endometrium. The possible beneficial effects of vitamins E and C on rat endometrium with long-term exposure to a 900-MHz EMF have not been reported previously. The experiment reported here sought to determine whether vitamins E and C have a preventive role in mobile phone-mediated oxidative stress and apoptosis in the endometrium. The duration of mobile phone use and its frequency are important factors. The exact duration also differs from one individual to another. Hence, setting the duration for experimental conditions is a complicated matter. In the present study, investigators chose the 30-min/d exposure because it is considered the mean exposure period of cellular phone use by most individuals. Frequency, the rate at which an EMF changes direction, is measured in Hz. One MHz is 1 million cycles per second. Analog telephones use frequencies between 800 and 900 MHz, similar to those used in the present study, and digital mobile telephones use frequencies between 1850 and 1990 MHz; microwave ovens use a frequency of 2450 MHz. Today's mobile telephones, with total power output of about 2W, are estimated to produce insignificant local heating, which is unlikely to produce any deleterious effects.

In previous studies, Ozguner et al^{21,23} showed that exposure to an EMF with a frequency of 900 MHz caused increased LPO in rat myocardium and retina. In addition, Yariktas et al³⁰ showed that exposure to an EMF increased LPO in rat nasal and sinus mucosa. Oktem et al²² determined that an EMF increased MDA levels in renal tissue and decreased superoxide dismutase, catalase, and glutathione peroxidase activities. However, it is not known whether LPO was caused by an EMF in the rat endometrium. The present study shows that exposure to an EMF with a frequency of 900 MHz has significant effects on rat endometrium, suggesting that ROS were generated under the experimental conditions employed. The change in MDA levels reported in the current study was regarded as an indicator of increased ROS production that occurred during the exposure period and may reflect the pathologic process of the exposure. These results support the hypothesis by which LPO has been suggested as one of the molecular mechanisms involved in EMF-induced damage.

Vitamin E, the most important lipophilic antioxidant, resides mainly in the cell membranes, thus helping to maintain membrane stability.³¹ Vitamin C is the most important hydrophilic free radical scavenger in extracellular fluids; it traps radicals in the aqueous phase and protects biomembranes from peroxidative damage.³² In addition to providing its antioxidant effects, vitamin C is involved in the regeneration of tocopherol from tocopheryl radicals in the membrane. Thus, vitamins E and C may have interactive effects.³³

Vitamins E and C reduce MDA in many organs. Many clinical and research centers are investigating the usefulness of antioxidant supplementation and its role in the prevention of preeclampsia. Because oxidative stress results in luteolysis, antioxidant supplementation, for example, of vitamins E and C has been shown to have beneficial effects in preventing luteal phase deficiency and the resulting increased pregnancy rate.^{34,35} Improved pregnancy rates were also reported with combination therapy with the antioxidant pentoxifylline and vitamin E supplementation given

over 6 mo to patients with thin endometria who were undergoing in vitro fertilization with oocyte donation.³⁶ Comparison of melatonin with other indolamines and the well-known antioxidant vitamin E has been reported.^{37,38} Ozguner et al^{20,21,23} showed that combined and single use of melatonin and caffeic acid phenethyl ester can reduce LPO caused by EMF in rat heart, kidney, and retina. Results of the current study show that a combination of vitamins E and C can reduce LPO caused by an EMF in rat endometrium; this supports the findings of previous studies.

Recent data show the controversial effect of a high-frequency EMF on cell proliferation and apoptosis, both of which are sensitive to environmental stresses.^{39,40} A possible effect of EMFs on DNA damage seen as single- and double-strand breaks in rat brain cells was reported.⁴¹ An increase in the incidence of micronucleus formation in the lymphocytes of workers exposed to microwave radiation has also been found.⁴² Merola et al⁴³ showed that 900-MHz radiofrequency exposure was unlikely to elicit a general stress response in neural cells; they found that neural cells were able to compensate for any effect without altering their normal physiology. Tarantino et al⁴⁴ reported a reduced apoptotic index in the liver and spleen but an increased apoptotic rate in the brains of rabbits exposed to a 650-MHz EMF. Capri et al⁴⁵ studied cell cycle phases, mitochondrial membrane potentials, and susceptibility to apoptosis in peripheral blood mononuclear cells from healthy donors who were exposed to a 900-MHz GSM (Global System for Mobile communication modulations) and found these parameters to be unaffected. In another study, Capri et al⁴⁶ found that 1800-MHz radiofrequency mobile phones (different GSM) did not affect apoptosis and heat shock protein 70 level in peripheral blood mononuclear cells from young and old donors. Pirozzoli et al⁴⁷ showed the effects of 50-Hz EMF exposure on apoptosis and differentiation in a neuroblastoma cell line; interference with regulation of the onset of differentiative and apoptotic processes of actively proliferating cells was noted.

The antiapoptotic activity of vitamins E and C has been reported by several authors.¹⁷ These antioxidant vitamins directly inhibit free radical-mediated apoptosis through elimination. The antioxidant vitamins E and C maintain the Bcl-2 protein in its functional form through their membrane-stabilizing action and thus inhibit the release of cytochrome C from mitochondria. Vitamins E and C can decrease the levels of tumor necrosis factor (TNF)- α in human monocytes and enhance the function of Bcl-2 in human endothelial cells. Modulation of the activities of TNF- α and Bcl-2 by vitamins E and C further decreases the activation of caspase-3 and, subsequently, DNA fragmentation.¹⁸

Recent studies have shown that Bcl-2 immunolabeling was predominant in glandular cells, peaking at the end of the follicular phase and disappearing at the onset of secretory activity; this indicates that apoptosis is normally upregulated during implantation in both rats and humans.⁴⁸ The stroma also displayed cyclic variations in Bcl-2 expression. Moderate expression of Bax in the proliferative endometrium and its increase in the secretory endometrium have been observed.

In the present study, investigators semiquantitatively studied tissue localization of apoptosis-related caspase-3, caspase-8, Bcl-2, and Bax through immunohistochemistry; they explored the significance of these and their involvement in the induction of endometrial apoptosis and in the ameliorating effects of vitamins E and C. A significant difference in immunolabeling of caspase-3 and caspase-8 was seen in

the endometrial epithelium and the stroma between samples obtained from the control and EMF groups. When tissue sections were treated with an antibody against Bcl-2, positive labeling was observed in the EMF group, but not in the control group. Immunohistochemistry data have shown that the distribution and immunoreactivities of Bax were significantly different between control and EMF group endometrial samples. Therefore, in the EMF group, the balance between Bcl-2 and Bax shifted toward death. Administration of vitamins E and C, along with exposure to an EMF, significantly reduced the extent of apoptosis.

In conclusion, in view of previous observations and current data, the potent free radical scavenger and antioxidant agents vitamins E and C seem to be highly promising agents for protecting endometrial tissue from oxidative damage and preventing apoptotic changes caused by a 900-MHz EMF exposure. However, additional studies with different frequencies and exposure periods are needed to prove the protective effects of vitamins E and C on oxidative stress and apoptosis in the endometrium caused by an EMF.

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