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Preliminary evidence suggesting that nonmetallic and metallic nanoparticles devices protect against the effects of environmental electromagnetic radiation by reducing oxidative stress and inflammatory status

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Abstract

Introduction: There is increasing interest in evaluating the potential health risks and biologic effects of exposure to extremely low-frequency magnetic fields (ELF-MF) and electromagnetic radiation (EMR), like those associated with personal computers, cellular phones, and environmental radiation (e.g., cellular towers, high-voltage power lines, radar). ELF-MF may generate free radicals in biological organisms, which leads to hyperoxidative status. Here, we investigated the potential efficacy of protective devices constructed with nonmetallic and metallic nanoparticles, which are conductors and semiconductors of electromagnetic energy.

Methods: In a before and after study, 20 healthy subjects who regularly used cellular phones and were exposed to typical environmental EMF were given one of three different (ELF-MF) protective devices. Blood samples were drawn at baseline and one month after using the devices to examine redox and inflammatory status.

Results: We found that, 30 days after using the devices, plasma levels of lipid peroxidation, nitrites, and interferon- γ decreased significantly. Furthermore, the disulfide glutathione/glutathione ratio decreased, which indicated reduced intracellular oxidative damage. These data suggested that continuous use of devices that contain nonmetallic and metallic nanoparticles could protect healthy subjects from EMF-induced oxidative/inflammatory damage.

Conclusion: Thus, for the first time, we showed that the devices tested could be useful in counteracting the deleterious effects of EMF pollution by neutralizing harmful radiation before it reaches the body.

Keywords: non-ionizing radiation, nanoparticles, mobile phone and computer users, oxidative stress, inflammation, grapheme

1. Introduction

During the last two decades, it has been reported that extremely low frequency magnetic fields (ELF-MF) may initiate a number of biochemical and physiological alterations in the biological systems of different species [1, 2]. Some authors reported a possible association between ELF-MF exposure and malignancy in children, especially leukemia, and cardiovascular and neurodegenerative diseases in adults [3-5]. A large number of *in vitro* studies have shown that ELF-MF affected cell proliferation [6], protein synthesis [7], and RNA/DNA synthesis [8]. Nevertheless, the genotoxicity of ELF-MF remains unclear. All studies have agreed that the deleterious effects of EMF exposure on living organisms depend on the frequency and density of the field and the time of exposure. Some authors have concluded that genotoxicity after exposure to EMF is secondary to other mechanisms, such as the production of free radicals [9-11].

It was suggested that a possible biological response to EMF exposure is the formation and prolonged survival of reactive oxygen species (ROS) and other free radicals [12]. Exposure to EMF caused overproduction of free radicals in the hearts and plasma of rats, which injured cellular functions and, eventually, caused cell death [13]. Moreover, EMF exposure reduced the efficacy of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), melatonin and the enzymes involved in the glutathione redox cycle [14]; those conditions resulted in free radical formation [15, 16]. An increasing number of subjects have reported subjective symptoms and hypersensitivity to a wide variety of electromagnetic sources, including radio and TV broadcasting stations and computer monitors [17]. Thus, sustained exposure to these and other EMF sources throughout life might explain, at least in part, the remarkable fall in antioxidative enzymatic activities observed in aged animals compared to young animals [14]. The potential association between EMF

exposure and many neurodegenerative diseases may also depend on the time of exposure to EMF [4, 18].

Cellular phones produce electromagnetic radiation (EMR). During a call, the antenna of a cellular phone emits radiofrequency electromagnetic fields that can penetrate 4-6 cm into the brain [19]. Even when not calling, cell phones emit a regular pulse of EMR when they are switched on [20]. An extensive number of experimental studies have indicated that pulsed digital radiation from cellular phones induced an array of biological impacts, including blood-brain barrier disruption, liver damage, and eye damage [21-23]. Specifically, a relationship has been proposed between intensive cell phone use and an increased risk of brain cancer [23]. Thus, several *in vivo* and *in vitro* models have been studied to examine links between EMFs, mutagenesis, and the mechanisms of cancer risk. In addition, EMR induces ROS formation; this causes an imbalance between oxidants and antioxidant defenses, which results in increased cell death with increased exposure time [24].

Emerging evidence has indicated that the radiation, emitted from cellular phones and other devices commonly used during daily life and occupational activities, is closely related to oxidative stress. Therefore, this study aimed to evaluate whether devices composed of nonmetallic and metallic ions that neutralize EMF and/or EMR might reduce oxidative/nitrosative stress in healthy subjects exposed daily to environmental EMF and EMR.

2. Methods

2.1. Subjects

The study included 20 healthy workers recruited from a nursing home, aged 30-50 years, who took no prescribed medications; had no neurological or psychiatric disorders, and had no cancer diagnosis. All workers had similar radiation exposure conditions. All

volunteers used cellular phones regularly, and they were exposed to environmental electric and magnetic fields. Blood samples were collected before and after use of devices. Informed consent was obtained from all participants in the study, which was approved by the Ethics Committee of Granada's University and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

For this study, the volunteers were provided each one with three devices (all from Pranan Technologies Ltd., Pamplona, Spain): the Pranan Phione, designed to shield the user from the EMF/EMR of cellular phones; it was stuck on the back of the phone unit; the Pranan Vitalizer 8-V-11 and the Pranan Relax 8-R-5, both designed to neutralize environmental EMF; the former was carried in the pocket and/or handbag of the study subjects, and the second device was placed under the bed pillow at night. The devices were small and easy to use. According to the manufacturer, the composition of the three devices was similar. Three devices were designed to ensure that the subjects carried at all times and were protected during all the day under any circumstances: mobile phone, computer, environmental EMF, etc.

These devices were based on structures made of nonmetallic nanoparticles (minerals) (1-200 nm) and metallic nanoparticles (1-200 nm) that act as conductors and semiconductors of electromagnetic energy. The nonmetallic nanoparticles contained graphene and silicon; the metallic nanoparticles contained gold, copper, and silver. Each of the three devices was designed for use in a different environment.

2.2. *Samples*

Blood samples were obtained from the antecubital vein of all subjects at 9:00 am, before and 30 days after using the three devices. Samples were centrifuged at $3000 \times g$ for 10

min at 4°C. Plasma aliquots were frozen and stored at -80°C until biochemical assays were performed. Erythrocytes were washed twice with cold saline, and aliquots were frozen at -80°C. On the day of the experiment, washed erythrocytes were hemolyzed in phosphate buffer (10 mM sodium phosphate, 1 mM ethylene diamine tetraacetic acid disodium [EDTA-Na₂], pH 6.25). Then, samples were deproteinized with ice-cold 10% trichloroacetic acid, and centrifuged at 20,000 ×*g* for 15 min. Supernatants were used for the measurements.

Urine was collected from each subject between 12 pm and 8 am. Diuresis was measured, and 10 ml aliquots of urine were frozen at -80°C until use for determining 6-sulfatoxymelatonin content, the main hepatic metabolite of melatonin, which is excreted in the urine, and serves as an index of the endogenous melatonin production.

2.3. Lipid peroxidation assay (LPO)

Plasma samples were thawed and centrifuged at 5000 ×*g* for 5 min. Supernatant samples (200 µl) were used to measure lipid peroxidation (LPO) content. LPO was measured with a commercial LPO assay kit that estimated both malondialdehyde (MDA) and 4-hydroxyalkenals (4HDA; Bioxytech LPO-568 assay kit; OxisResearch, Portland, OR, USA) [25]. LPO concentrations are expressed in µmol/l.

2.4. Nitrite plus nitrate determination (NO_x)

The concentration of nitrites in plasma was measured according to the Griess reaction, which converts nitrite into a colored azo compound. The color was detected at 550 nm on a spectrophotometer [26]. Plasma levels of nitrite plus nitrate (NO_x) are expressed in µmol/l plasma.

2.5. Glutathione (GSH) and glutathione disulfide (GSSG) assays

Erythrocyte glutathione (GSH) and disulfide glutathione (GSSG) levels were measured with an established fluorometric method [27] in a plate-reader spectrofluorometer (Bio-Tek Instruments, Inc., Winooski, VT, USA). Glutathione concentrations were calculated according to standard curves previously prepared. Levels are expressed in $\mu\text{mol/g}$ hemoglobin (Hb).

2.6. Glutathione peroxidase (GPx), Glutathione reductase (GRd), and Superoxide dismutase (SOD) assays

Erythrocyte levels of glutathione peroxidase (GPx) and reductase (GRd) activities were measured [28] in a UV spectrophotometer (model UV-1603, Shimadzu Deutschland GmbH, Duisburg, Germany). GPx and GRd activities are expressed in $\mu\text{mol/min} \times \text{g Hb}$. Superoxide dismutase (SOD) activity was measured in washed, hemolyzed erythrocytes, according to the method of Misra and Fridovich [29]. SOD activity was expressed in adrenaline units (U/g Hb).

2.7. Plasma cytokine assay

The multiplex human cytokine immunoassay kit (Millipore Corp., Billerica, MD, USA) was used to profile expression of inflammatory mediators (IL-1 β , IL-2, IL-5, IL-6, IL-8, IL-10, TNF- α , and INF- γ). The assay was performed according to the manufacturer's instructions. A standard curve was constructed for each cytokine. Levels are expressed in ng/l.

2.8. Statistical analysis

Data are expressed as the mean $\pm$ SEM. ANOVA, followed by the Student's *t*-test, were used to compare means between groups (samples prepared before and after the use of the devices). A *P*-value less than 0.05 was considered statistically significant.

3. Results

Given that the sample was small the results in Figures 1 to 4 represent the mean values of the data obtained after combining data from all the three devices during 30 days in the 20 studied subjects. The comparative effectiveness of each device was not separately assessed, because the rationale of the study was to evaluate the 24-hour protection with the devices.

Plasma LPO, a marker of oxidative damage to cell membranes, decreased significantly after using the devices for 30 days compared to baseline values (before using the devices; Fig. 1; $P < 0.01$). Also, the GSSG/GSH ratio, an index of intracellular oxidative damage, was significantly reduced at the end of the study (Fig. 2; $P < 0.05$). The total glutathione (GSH+GSSG) levels did not change during the study. The minor changes observed in the GSH redox cycle could be explained by important changes in the activity of both GPx and GRd, which increased significantly with the use of devices, and maintained glutathione homeostasis (Fig. 3A and B, $P < 0.001$). Erythrocyte SOD activity, however, decreased significantly in subjects after using the devices (Fig. 3C, $P < 0.05$).

We measured the plasma levels of the inflammatory marker, NOx, which reflects the production of nitric oxide (NO $\bullet$) and, subsequently, the changes in inducible nitric oxide synthase (iNOS) activity and/or expression. We also measured the plasma levels of pro- and

anti-inflammatory cytokines. Our data showed a significant reduction in the inflammatory response after using the devices. We observed reductions in both NO_x (Fig. 4B, $P < 0.01$) and INF γ (Fig. 4A, $P < 0.001$) levels. The other cytokines assayed (IL-1 β , IL-2, IL-6, IL-8, and TNF α) and the anti-inflammatory cytokine, IL-10, did not show significant changes (data not shown).

The levels of plasma melatonin and the urinary excretion of its main metabolite, 6-sulfatoxymelatonin, remained unmodified throughout the study (data not shown). No changes in the morning plasma cortisol were found (data not shown).

4. Discussion

This study is the first to show that devices that contained nonmetallic nanoparticles (graphene and silicon) and metal nanoparticles (gold, copper, and silver) may reduce oxidative stress and inflammatory status in healthy subjects. We evaluated three devices, which were designed for different environments. All three devices could neutralize ELF-EMF. It is well known that gold is diamagnetic, due to its counteraction of the paramagnetic behavior of conduction electrons by orbital and ionic core diamagnetism. Chemically-induced permanent magnetism could also be extended to Ag and Cu [30]. Furthermore, it is well known that metallic ions can be intersubstituted to tune or transform electronic behavior [31-35]. Moreover graphene, despite its single-atom thickness, can guide electromagnetic fields with properties that depend on both the geometry of the graphene sheet and the external electrical potentials. Graphene and related two-dimensional crystals may play a major role in future energy conversion [36]. Therefore, these devices might act as passive converters of non-ionizing waves, which can alter the bioenergetic field of the cells in the human body [37]. It remains to be clarified whether the reduction in oxidative/nitrosative and inflammation

markers observed in the present study was due to a counteraction of environmental EMF and/or a counteraction of cellular phone EMR. This is a preliminary study and additional experimental approaches are required to investigate the mechanism(s) of action of these devices and whether one device is more effective than another.

Our finding that these devices could reduce and/or neutralize damage caused by EMF and EMR could lead to interesting applications for preventing potential ELF-MF-dependent health risks. An important step forward in that direction was taken by the International Agency for Research on Cancer, which classified ELF-MF as a suspected carcinogen [38]. Moreover, ELF-MF exposure was also related to many other pathologies, including Alzheimer's disease, in subjects with occupational exposure to ELF-MF [39]; neurobehavioral disorders, in adult mice that were exposed as a fetus to radiofrequency radiation from cellular phones [40]; and stress-based cornea and lens pathologies, in subjects exposed to personal computer monitor radiation [41].

On the other hand, although the cellular mechanisms are unclear, most studies have suggested that EMF and EMR had deleterious effects on human and animal physiology, and oxidative stress was the underlying pathogenic mechanism [13, 42]. It is well known that ELF-MF exposure increases mitochondrial production of ROS [14]; indeed, mitochondrial dysfunction and subsequent bioenergetic failure represent a common pathogenic mechanism of many diseases and aging [43-45]. The present study demonstrated that, after 30 days of using these devices, normal subjects exhibited a significant reduction in blood oxidative stress markers.

Among oxidative stress markers, LPO is commonly used as an index of free radical damage to cell membranes, because free radicals cause peroxidation of unsaturated fatty acids [31]. Here, we showed that using the test devices for 30 days caused a significant reduction in LPO; this finding could be related to a reduction in the ELF-dependent oxidative stress [13]. The efficacy of the test devices was further supported by the GSSG/GSH ratio reduction in erythrocytes, the best index of intracellular redox status. Of interest, there were also minor

changes in GSH and GSSH levels, which can be explained by the GPx and GRd activities, the primary enzymes in the GSH cycle. The increased GPx activity observed after using the test devices reflected an improvement in peroxide detoxification by the erythrocytes. The increased GRd activity observed after using the test devices was responsible for the recovery of the GSH levels from GSSG, which maintained the intracellular pool of GSH. Consequently, the efficiency of the GSH redox cycle was significantly improved with the test devices. These results are of great significance because, in the absence of an adequate GSH pool, the ability of the intracellular antioxidative system is severely disrupted, which leads to a hyperoxidative state, cellular dysfunction, and cell death [46].

The observed decrease in SOD activity probably reflected a lower degree of oxidative stress after 30 days of using the test devices. Some authors have proposed that the changes caused by ELF-MF in oxidative status represent an early response, and that no experimental findings have provided a mechanistic explanation for their deleterious effects [47]. Other authors [48] have demonstrated that ELF-MF from cellular phones can damage sperm viability through mechanisms involving electron leakage from the mitochondria and generation of oxidative stress. Other authors have found a disruption in the oxidant/antioxidant balance in the eye and other tissues exposed to EMR from cellular phones [49, 50]. Disruptions in the redox balance between ROS and antioxidants can tip the equilibrium toward a hyperoxidative condition [46, 51]. Thus, ELF-MF exposure may represent a risk factor for the occurrence of oxidative stress-based pathologies, and devices like those tested here could be useful in counteracting oxidative stress.

On examining the inflammatory status, we found that NO• and INF γ decreased significantly throughout the study. NO• is a free-radical gas that has both beneficial and deleterious properties. NO• can function as an anti-inflammatory/antioxidant and even a cytoprotective molecule at low concentrations, like the NO• produced by constitutive endothelial and/or neuronal nitric oxide synthases. However, in inflammatory conditions, induction of the iNOS yields high amounts of NO• that become cytotoxic, mainly due to the inhibition of mitochondrial respiration and the nitration/nitrosylation of proteins [52, 53]. TNF α and INF γ stimulate the expression of iNOS; thus, these cytokines play an important role in the regulation of NO• in many diseases [52]. In addition to the reduction in NO•, we found a significant reduction in INF γ levels. This finding suggested that the inflammatory status declined as a consequence of using the test devices for 30 days.

5. Conclusions

Although we did not quantify the levels of ELF-MF exposure in this study, we showed that the use of specific devices significantly reduced the oxidative and inflammatory status in a group of normal subjects that were exposed to environmental EMF, due to the regular use of cellular phones. Our results suggested that the devices tested here could protect against EMF pollution by rectifying the damaging EMF forces to maintain the health of the body.

However, this is a preliminary study, and considering that the subjects are normally exposed to long term of EMF and EMR, it will be interesting to have results after long term exposure. Several studies have found that exposure to ELF-MF represented a potential health risk. Although the cellular mechanisms remain unclear, it has been suggested that oxidative stress could be a key factor in the pathogenicity of ELF-MF exposure [54]. Some limitations to the present study included the lack of comparisons between exposure with non exposure to electromagnetic radiation and the relative amounts of phone and/or computer use among the participants; so, future studies will be required to address these questions. Anyway, until definitive scientific answers are available, the adoption of preventive actions is advisable [23, 55].

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Conflict of interest

The authors declare they have no competing interests

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Figure Legends

Figure 1:

Plasma lipid peroxidation (LPO) levels before (C) and 30 days after using the devices. **P < 0.01 for 30 vs. C.

Figure 2:

Erythrocyte levels of (A) GSH and (B) GSSG; and (C) the GSSG/GSH ratio measured before (C) and 30 days after using the devices. *P < 0.05 for 30 vs. C.

Figure 3:

Erythrocyte activities of (A) GPx, (B) GRd, and (C) SOD, before (C) and 30 days after using the devices. **P < 0.01 for 30 vs. C.

Figure 4:

Changes in plasma (A) INF γ and (B) NOx levels, before (C) and 30 days after using the devices. **P < 0.01 for 30 vs. C.

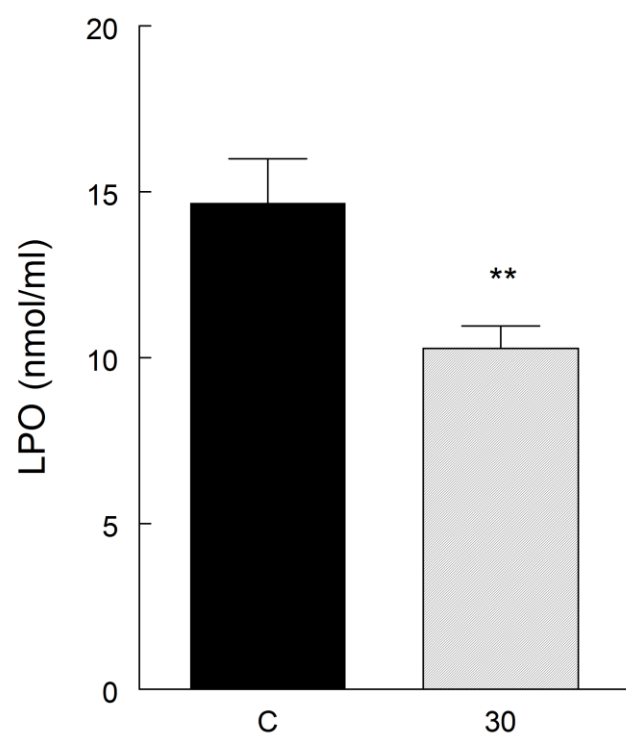
Figure 1

Figure 2

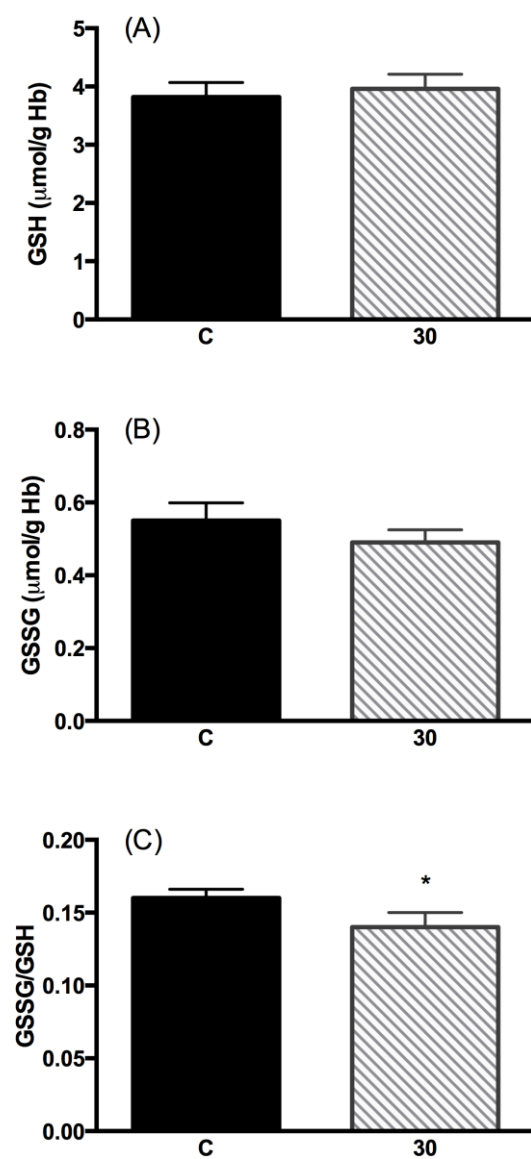


Figure 3

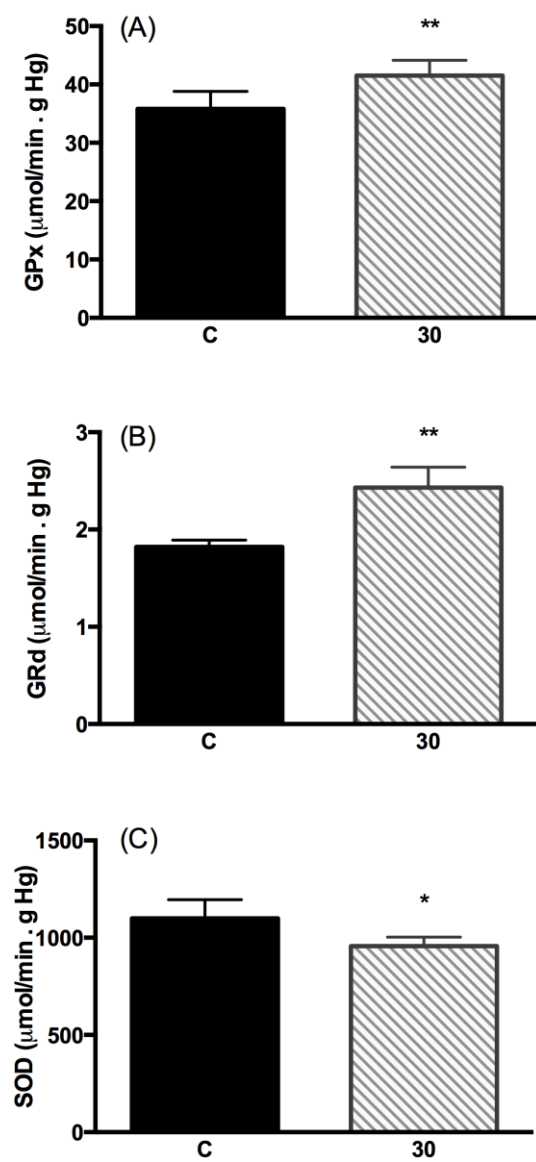


Figure 4