Electromagnetic fields at 2.45 GHz trigger changes in heat shock proteins 90 and 70 without altering apoptotic activity in rat thyroid gland

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Summary
Non-ionizing radiation at 2.45 GHz may modify the expression of genes that code for heat shock proteins (HSP) in the thyroid gland. Using the enzyme-linked immunosorbent assay (ELISA) technique, we studied levels of HSP-90 and HSP-70. We also used hematoxilin eosin to look for evidence of lesions in the gland and applied the DAPI technique of fluorescence to search for evidence of chromatin condensation and nuclear fragmentation in the thyroid cells of adult female Sprague-Dawley rats. Fifty-four rats were individually exposed for 30 min to 2.45 GHz radiation in a Gigahertz transverse electromagnetic (GTEM) cell at different levels of non-thermal specific absorption rate (SAR), which was calculated using the finite difference time domain (FDTD) technique. Ninety minutes after radiation, HSP-90 and HSP-70 had decreased significantly ($P<0.01$) after applying a SAR of 0.046±1.10 W/Kg or 0.104±5.10^{-3} W/Kg.

Twenty-four hours after radiation, HSP-90 had partially recovered and HSP-70 had recovered completely. There were few indications of lesions in the glandular structure and signs of apoptosis were negative in all radiated animals. The results suggest that acute sub-thermal radiation at 2.45 GHz may alter levels of cellular stress in rat thyroid gland without initially altering their anti-apoptotic capacity.

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Key words: Apoptotic activity, Electromagnetic fields, Shock proteins, Thyroid gland

Introduction
Over the last decade, numerous scientific studies have described changes in biological parameters after interaction with electromagnetic fields (EMFs) in living organisms. People are generally and daily exposed, silently and continuously, to numerous sources of non-ionizing radiation. Due to individual susceptibility or work exposure, certain segments of the population may be more susceptible to possible adverse effects.

Numerous epidemiological and experimental studies have been carried out to evaluate the risk of cancer (Wakeford, 2004), effects on the nervous system (Regel and Achermann, 2011), hematological effects (Jin et al., 2011), effects on metabolism or the endocrine system (Karasek and Woldanska-Okonska, 2004) and effects on the general population or groups directly at risk from EMF-related exposure.

Heat shock proteins (HSPs) are found in all living organisms, from bacteria to humans (Morimoto et al., 1992). They participate in cellular-physiological processes of hormonal signaling, cellular cycle control and cellular proliferation and differentiation (Morimoto et al., 1992; Helmbrecht et al., 2000). They also help regulate and control cellular environmental stress processes (Gupta et al., 2010; Trautinger et al., 1996) related to the etiopathology of various diseases (Kalmar and Greensmith, 2009). Concern regarding potential risks of human exposure to EMFs has led to the creation of numerous experimental models for laboratory detection of biomarkers that are sensitive to this physical stimulus. Evidence has been found for changes in the expression of HSPs in various models of human cell lines (Lee et al., 2005; Caraglia et al., 2005; Alfieri et al., 2006) or in vivo rat tissue (Jorge-Mora et al., 2010) after exposure to experimental radio frequency systems. Other studies report no apparent changes in these biological markers after applying other experimental models in the laboratory (Wang et al., 2006; Chauhan et al., 2006; Valbonesi et al., 2008).

Recently, numerous studies have appeared describing the relationship between exposure to electro-magnetic sources, such as extremely low frequency (ELF) EMFs and radio frequency (RF), and various thyroid gland pathologies such as cancer (Milham and Morgan, 2008), alterations in the production of thyroid hormones (Rajkovic et al., 2003; Koyu et al., 2005; Esmevka et al., 2010) and other dysfunctions (Bergamaschi et al., 2004). Cellular levels of HSP-90 and HSP-70 in the thyroid gland are known to be linked to homeostasis (Wallin et al., 1992) and levels of cytotoxicity in certain glandular pathologies (Samadi et al., 2009; Paggi et al., 1995). These proteins may be biomarkers for detecting toxicity or environmental stress that...
affects normal thyroid tissue functioning. In this study, we used levels of HSP-90 and 70 to analyze cellular stress induced by radiation (Jaroš and Lindquist, 2010), and how anti-apoptotic activity and integrity (Joly et al., 2010) are affected in female rat thyroid tissue exposed to 2.45 GHz radio frequency in an experimental GTEM system. We also used rectal temperature probes to measure body stress in animals, in order to determine if there was any interaction between variations in the post-radiation temperature of the animals and cellular stress.

Materials and Methods

Animals
All experiments were carried out according to European regulations on animal protection (Directive 86/609), the Declaration of Helsinki and/or the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Santiago de Compostela.

Adult female Sprague-Dawley rats were used in the study. The rats weighed 230–250 g, were housed in individual cages with free access to food and water and were maintained at ±2°C under a 12:12 h light/dark regime. There is already evidence that estrogen may act directly in female rat thyroid cells to modulate their proliferation and functioning (Santin and Furlanetto, 2011).

Experimental design
A total of 96 female Sprague-Dawley rats were used and distributed equally into the following groups:

Group I (n=48): The rats were divided into 4 subgroups (n=6); each rat was exposed to 30 min* of microwave radiation at different levels: 0 (control), 1.5, 3.0 and 12 W **. The rats were kept alive for 90 min***, then slaughtered and perfused with fixative.

Group II (n=48): The rats were divided into 4 subgroups (n=6); each rat was exposed to 30 min of microwave radiation at different levels: 0 (control), 1.5, 3.0 and 12 W **. The rats were kept alive for 24 h***, then slaughtered and perfused with fixative.

**The minimum time exposure was tested previously.

***90 min post-radiation was chosen as the moment of slaughter for Group I animals because peak expression of the HSP-90 and 70 proteins occurs at that time. Group II animals were examined 24 h after exposure to radiation, which is the generally-recognized recovery interval.

Experimental radiation system protocol and description of the numerical simulations by FDTD SAR estimation

The power was delivered by a controlled signal during the radiation procedure. The amplifier (AMP) output was connected to the directional coupler (DC) that fed the gigahertz Transverse Electromagnetic cell (Schaffner GTEM 250 1.25 m x 0.65 m x 0.45 m). The rat (R) was placed inside the cell and positioned in the region of maximum field uniformity.

The DC makes it possible to measure the input power (PIN) and reflected power (PREF) values. The PIN was read by a power meter that also monitored the purity of the input signal; while the PREF value was obtained directly by means of a spectrum analyzer. All the measuring instruments were certified and calibrated by Agilent Technologies. This exposure setup provides good field uniformity and the behavior of a traveler wave can be simulated. The system components and operation were described in detail by Jorge-Mora et al. (Jorge-Mora et al., 2010).

The specific absorption rate (SAR) values were estimated with the aid of SEMCAD X (Schmid & Partner Engineering AG, Reference manual for the SEMCAD simulation platform for electromagnetic compatibility, antenna design and dosimetry). All the SEMCAD simulation models were calibrated with the aid of SEMCAD simulation software. The Sprague-Dawley model R5 numerical (voxel) phantom rat (GTEM 250 1.25 m x 0.65 m x 0.45 m) was used, weighing 198.3 g and composed of 60 different tissues assembled in 1.15 mm thick slices (tissue morphologies obtained by magnetic resonance images). The numerical phantom was radiated by a plane wave impinging its left side, with the magnetic field H parallel to its main axis (Jorge-Mora et al., 2010).

The electrical field value E was determined by Eqn 1:

\[
E = \sqrt{Z_{0}P_{m}/(k^{2}c^{2})}
\]

where k is the septum height in the exposure zone (position of the MH), P_{m} \xi is the input power of the GTEM cell, P_{R} = P_{m} - P_{RFS}, Z_{0} = 50 \Omega is the input impedance of the cell, and \xi is the coefficient that depends on the ripple field in the position MH, which is considered to have a value of 2 (Schaffner Electrotest GmbH GTEM Test Cells, Datasheet 2005).

The SARs were estimated by applying a correction factor to the values obtained from the numerical simulations, in proportion to the ratio between the weight of the model rat and the weights of the experimental rats, as specified by the following expression:

\[
SAR_{E} = SAR_{R} \times W_{E}/W_{R}
\]

where SAR_{E} is the estimated value of the experimental SAR, SAR_{R} is the SAR obtained during the simulation, W_{E} = 198.3 [g] (the weight of the model rat) and W_{R} [g] is the weight of the experimental rat.

Stress levels and changes in rectal temperature after radiation

Using a Eutech digital thermometer, animal temperatures were measured before placement in the radiation chamber, and again at 0, 30, 60, 90 min and 24 h after radiation. Monitoring the rectal temperature of radiated and non-irradiated rats made it possible to determine temporal variation in levels of body stress (Dallmann et al., 2006), as well as differences in responses among the animals in the experiment.

Tissue extraction and preparation of cell extracts

A total of 24 animals were used for the ELISA technique. Once the 90 min and 24 h time periods had elapsed after radiation, the animals were thoroughly anesthetized with ethyl ether and the thyroid gland tissue was extracted under a microscope with a Nikon Eclipse CF160 optical system. The animals were subsequently slaughtered. The extracted thyroid glands were stored at -30°C for use.

Enzyme-linked immunosorbent assay (ELISA)

The glands were removed from cold storage and tissue lysis was carried out using the ProteoJet Mammalian Cell Lysis Reagent kit (Fermentas), following manufacturer’s instructions. Then, the concentration of the protein in the extracted tissue of each sample was quantified using the Bio-Rad Protein Assay kit (BioRad Laboratories), with bovine serum albumin (BSA) as the standard protein.

To each ELISA polystyrene sample plate well (Iwaki), 1 µg of each sample was added and then incubated overnight at 4°C in 100 µl/well of coupling buffer (Na2CO3 0.015 M, HNaCO3 0.035 M, pH 9.6). Then, the plates were blocked for 2 h at room temperature using Tris buffer saline (TBS: Tris 50 mM, NaCl 0.15 M, pH 7.2) containing 0.2% Tween 20 and 5% skim milk. After washing with PBS we added a 1/20 diluted solution of anti-HSP-90 and anti-HSP-70 monoclonal antibody (Santa Cruz Biotechnology) and this was incubated for 1 h at 37°C. After several TBS rinses, the samples were placed in a 1/200 diluted solution of anti-IL-2 (polyclonal rabbit antibody (Dakopatts) and incubated for 1 h at 37°C. Finally, the plates were washed with TBS and then a 0.04% ortho-phenylenediamine (Sigma) substrate was added in phosphate-citrate buffer (Na2HPO4 0.2 M, citric acid 0.1 M, pH 5.0) and 0.001% H2O2. The reaction was stopped after 20 min incubation and H2SO4 3 N was added. The optical densities (OD) were then read in ELISA microplate reader at 492 nm. A qualitative relationship was established in which absorbance was proportional to the concentration of protein detected in 1 µg/ml.

Two samples of thyroid tissue were extracted from each animal and the technique was replicated.

Tissue extraction and preparation of morphological techniques

After radiation, the rats were left to rest for 90 min or 24 h and then slaughtered for tissue extraction. The slaughter procedure involved an intraperitoneal injection of sodium pentothal to diminish stress levels, followed by an overdose of ether just prior to slaughter. The tissues were fixed in paraformaldehyde for 24 h (10% in buffer phosphate), rinsed in alcohols and toluene, placed in a block of paraffin and cut into sections 5-6 microns thick. Three sections of each animal from each group (2) and the four subgroups (6 animals per subgroup) were used for each morphological technique described as follows (H&E and DAPI).

Hematoxylin-eosin (H&E) staining

The sections were then placed in xylol for 15 min, after which tissue was then hydrated alternately in pure 96% and 70% alcohol for 5 min periods, with a final rinse in distilled water. Hematoxylin tincture was then applied for 5 min, followed by a final 3 min rinse in water. Eosin tincture was applied for 1 min and then dehydration was carried out with 70%, 96% and 100% alcohol. Finally, the samples were submerged in xylol and mounted. The samples were examined under a conventional optical microscope to look for signs of lesions in the follicular cells and connective or colloid tissue of the thyroid gland.
DAPI staining of nuclei
These sections were rinsed in PBS wash and incubated with 0.8 mg/ml of 40, 6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich) in PBS for 15 min at room temperature. After several washes in PBS, the slides were mounted in PBS/glycerin. DAPI-stained tissues were visualized by fluorescence microscopy.

Quantification and statistical analysis
An ELISA microplate reader at 492 nm was used to quantify HSP-90 and HSP-70. Quantification and statistical analysis

Levels of HSP-90 and HSP-70 determined by ELISA

Results
SAR
Table 1 shows the mean values of the average SAR in rat thyroid and body, as well as the mean values of the SAR per 1 g of thyroid and body, obtained from individual measurements of rats exposed to different powers (P) and electric fields (E). Using a one-way ANOVA test followed by a Holm-Sidak test, statistically significant differences were found among the SAR values measured for the various powers (1.5, 3 and 12 W) applied to the radiated subgroups and for peak SAR values in 1 g of rat thyroid or body (P<0.001, significant differences P<0.05). The increase in average and maximum SAR values is directly proportional to the initial power (P) and the electric field value (E) for each subgroup (Fig. 1).

Stress levels and changes in rectal temperature after radiation
The effects of the various power levels were not dependent on exposure time. There is no statistically significant interaction between power and time (P=0.886).

Comparisons of the power factor revealed that there were no significant temperature differences among the groups (1.5, 3 and 12 W) or with regard to the control group (0 W).

Time comparisons revealed significant differences in average temperatures before and after radiation (at 0, 30, 60 and 90 min P=0.00038, P=0.0020, P=0.0029 and P=0.015, respectively). There were no significant differences in mean temperatures after radiation and at 24 h (P=0.230). Significant differences (P=0.003) only appeared at maximum power (12 W) with respect to the group exposed for 0 min.

Table 1. SAR values in thyroids and bodies of the experimental rats, calculated from the power (P) and electrical field (E).

| Power (W) | Electric field (V/m) | SAR in 1 g thyroid (W/kg) | SAR in body (W/kg) | Peak SAR in 1 g body (W/kg) |
|----------|----------------------|--------------------------|-------------------|-----------------------------|
| 1.5      | 28.48                | 0.046±1.10*              | 0.0169±7.10*      | 0.089±9.10*                 |
| 3        | 40.28                | 0.104±5.10*              | 0.076±4.10*       | 0.180±9.10*                 |
| 12       | 80.56                | 0.482±12.10*             | 0.340±10.10*      | 0.795±2.10*                 |

Experimental measurement of specific absorption rate by FDTD

The effects of different power levels were found to depend on the time elapsed after radiation. There was a statistically significant interaction between power and time elapsed after radiation (P=0.018).

Comparisons of the power factor showed significant differences between the control subgroup (0 W) and groups exposed to 1.5 W (P=0.00008) or 3 W (P=0.0003), but not between the control and the group exposed to 12 W (P=0.0905).

The subgroup irradiated at minimum power (1.5 W) showed significant differences in HSP-90 levels at 90 min after irradiation, compared to levels at 24 h after irradiation (P=0.001) (Fig. 2A).

Group I (rats slaughtered and fixed 90 min after irradiation): The amount of HSP-90 expressed in the thyroid gland of rats radiated at 1.5 W and 3 W was significantly different from that found in non-irradiated rats (P<0.0001, P<0.022, respectively). Protein levels in rats irradiated at minimum power (1.5 W) also showed significant differences with respect to rats irradiated at 3 W or 12 W (P<0.009, P<0.0001). Protein levels in rats irradiated at maximum power (12 W) were not significantly different from those in the non-irradiated group (P<0.283) (Fig. 2A).

Group II (rats slaughtered and fixed 24 h after irradiation): The amount of HSP-90 expressed in the thyroid gland of rats exposed to radiation at 3 W was significantly different from that found in non-irradiated rats (P<0.003). However, there were no significant differences in HSP-90 levels in rats irradiated at 1.5 W or 12 W when compared to non-irradiated-rats (Fig. 2A).

Results for HSP-70
The difference in the mean values for each power level was statistically significant (P=0.037) in the thyroid gland, after allowing for the effects of time differences after radiation. The difference in the mean values for the times elapsed after radiation was not statistically significant (P=0.924).

The effect of different power levels was not dependent on the time elapsed after radiation. There was not a statistically

Table 1 shows the mean values of the average SAR in rat thyroid and body, as well as the mean values of the SAR per 1 g of thyroid and body, obtained from individual measurements of rats exposed to different powers (P) and electric fields (E). Using a one-way ANOVA test followed by a Holm-Sidak test, statistically significant differences were found among the SAR values measured for the various powers (1.5, 3 and 12 W) applied to the radiated subgroups and for peak SAR values in 1 g of rat thyroid or body (P<0.001, significant differences P<0.05). The increase in average and maximum SAR values is directly proportional to the initial power (P) and the electric field value (E) for each subgroup (Fig. 1).

Stress levels and changes in rectal temperature after radiation
The effects of the various power levels were not dependent on exposure time. There is no statistically significant interaction between power and time (P=0.886).

Comparisons of the power factor revealed that there were no significant temperature differences among the groups (1.5, 3 and 12 W) or with regard to the control group (0 W).

Time comparisons revealed significant differences in average temperatures before and after radiation (at 0, 30, 60 and 90 min P=0.00038, P=0.0020, P=0.0029 and P=0.015, respectively). There were no significant differences in mean temperatures after radiation and at 24 h (P=0.230). Significant differences (P=0.003) only appeared at maximum power (12 W) with respect to the group exposed for 0 min.

Table 1. SAR values in thyroids and bodies of the experimental rats, calculated from the power (P) and electrical field (E). The SAR values were compared by One-Way ANOVA differences between power (P) or electric field (E) and an a posteriori Holm-Sidak test. For multiple comparisons, differences were considered significant at P<0.05. *Indicates significant differences between \((P=1.5)^2\) \((P=3)^3\) \((P=12)\).
significant interaction between power and time after radiation \((P = 0.556)\).

Comparisons of the power factor showed significant differences between the control subgroup (0 W) and groups that received 1.5 W or 3 W \((P = 0.0098, P = 0.0149)\), but there were no significant differences with respect to the group exposed to 12 W \((P = 0.142)\).

Group I: The amount of HSP-70 expressed in the thyroid of rats exposed to radiation at 1.5 W was significantly different from that observed in non-irradiated rats \((P < 0.010)\). However, the HSP-70 levels in the thyroid of rats exposed to radiation at 3 W or 12 W were not significantly different from levels observed in non-irradiated rats \((P = 0.060, P = 0.156)\) (Fig. 2B).

Group II: Expression of HSP-70 in the thyroid of rats exposed to radiation at 1.5 W, 3 W and 12 W was not significantly different from levels observed in non-irradiated rats \((P = 0.065, P = 0.371\) and \(P = 0.50)\). See Fig. 2B.

H&E analysis
At 90 min and 24 h after radiation, examination of the sections tinctured with H&E under an optical microscope at 40× and 100× magnification did not show lesion alterations in the thyroid tissue structure that would affect the follicular cells, connective tissue or colloid tissue in the animals exposed to 1.5 W and 3 W when compared to the thyroid tissue of non-irradiated animals (Fig. 3A–D). At 90 min and 24 h after exposure to non-ionizing radiation exposure to 2.45 GHz, only a slight loss of cell cohesion was observed in some follicles (3E,F).

Results of DAPI staining
Fluorescence microscopy with a DAPI staining was used to examine the nuclear morphology of different cells for signs of apoptosis. No morphological changes, such as chromatin condensation or nucleus fragmentation, were observed in DAPI-stained sections from irradiated (all groups and different SARs) or non-irradiated animals. However, when the samples were compared to positive controls subjected to gamma radiation, we found positive signs of chromatin condensation 6 h after radiation (Fig. 4).

Discussion
We are not aware of any prior studies defining stress levels in thyroid gland cells that quantify HSP indices in live animals exposed to electromagnetic sources. The thyroid gland regulates immune functions and various hormones, so the possibility of non-ionizing radiation provoking changes in the machinery of the
chaperone cells of the gland constitutes a very relevant matter. Our results indicate important changes in HSP-90 and HSP-70 levels after exposure to 2.45 GHz electromagnetic fields. A significant decrease 90 min after exposure, when maximum values were reached in the control animals, occurs in HSP-90 and HSP-70 when the minimum SAR is applied (0.046 ± 1.10^2 and/or 0.104 ± 5.10^2). Furthermore, the thyroid tissue was found to be most resistant to recovery at radiation levels of 3 W. Twenty-four hours after radiation, the basal protein levels of exposed animals had still not returned to the levels of the control or non-exposed animals.

The 2.45 GHz frequency was used in this experiment based on biological effects described by other authors (Malyapa et al., 1998; Pauraj and Behari, 2006) and our own previous studies of HSP-90 in several regions of the brain (Jorge-Mora et al., 2010). This frequency is commonly used in telecommunications and for therapeutic purposes. At sub-thermal levels, in vitro human cell models showed significant changes in the expression of HSP-90 but not HSP-70 (Lee et al., 2005; Wang et al., 2006; Alfieri et al., 2006; Perez et al., 2008). However, other authors report changes in HSP-70 only when very high, thermal levels of 20 W/kg power were applied (Tian et al., 2002). The results of the present experiment indicate that low SAR levels (peak SAR...
0.089±9.10^-3 or 0.180±9.10^-3 in the body and 0.041±2.10^-3 or 0.076±4.10^-3 in the thyroid), cause decreases in levels of heat shock proteins in thyroid cells. There is a wide variety of environmental stimuli that can cause cellular stress and modify the synthesis of HSP-90 and/or HSP-HSP-70 in mammal tissue, significantly affecting the maintenance of cellular homeostasis (Morimoto, 1993; Kregel, 2002; Jarosz and Lindquist, 2010), anti-apoptotic processes and cellular differentiation (Schmitt et al., 2007; Laneau et al., 2007). The most well-known inductors are temperature (Kregel, 2002), toxic agents (Gupta et al., 2010) oxidative stress (Kalmar and Greensmith, 2009) and ultraviolet radiation (Trautinger et al., 1996) or ionizing radiation (Calini et al., 2003; Kang et al., 2002).

Although some authors describe what seems to be a cellular stress response, one would expect it to take the form of an increase in HSP expression rather than a decrease. De Pomerai et al., for example, later rectified their study, saying that what they had taken to be a ‘non-thermal’ effect (de Pomerai et al., 2000; de Pomerai et al., 2003) was in fact a thermal one (de Pomerai et al., 2006). Our experience, which has been corroborated by other studies, suggests that the interaction of RF in the tissues might activate thermal and non-thermal mechanisms simultaneously (Jorge-Mora et al., 2010; López-Martín et al., 2006) was in fact a thermal one (de Pomerai et al., 2006). Our experience, which has been corroborated by other studies, suggests that the interaction of RF in the tissues might activate thermal and non-thermal mechanisms simultaneously (Jorge-Mora et al., 2010; López-Martín et al., 2011; López-Martin et al., 2006; López-Martin et al., 2009; Curcio et al., 2005). In some cases one mechanism is more apparent than the other; in this experiment, rectal temperature was slightly higher in animals with higher SAR. However, this did not result in a significant increase in the expression of HSP-70 and 90, which is probably compensated by the thermoregulatory mechanisms of the animals (Table 2).

The decrease in heat shock proteins seems to indicate the existence of non-thermal physical stimuli acting by unidentified mechanisms via low-intensity electrical fields, with no direct relationship between power and magnitude of effect. Because the animals received non-ionizing radiation to the entire body through the skin, the system is subject to multiple physical stimuli with biological repercussions that are difficult to identify precisely. Tissue stress can be added to other mechanisms induced by the simultaneous action of different functional circuits (Jorge-Mora et al., 2011).

The literature also describes decreases in HSP-70 and 90 twelve hours after a state of anoxia (Ramaglia and Buck, 2004) and during cerebral ischemia (Yang et al., 2005). These data indicate that increased HSP expression is not critical in early adaptation. However, late regulation involving the increase of heat shock proteins suggests that stress proteins play a role in promoting long-term tolerance.

The decreased levels of HSP-90 in the thyroid gland after radiation are much lower than those required for normal cellular functions. In a stressful environment this protein can destabilize and alter the relationship between genotype and phenotype (Jarosz and Lindquist, 2010). HSP-90 expression in the thyroid gland varies significantly; in normal tissue the protein levels are much higher than in the hyperplastic gland or in papillary cancer of the thyroid (Wallin et al., 1992). Various authors relate this to cellular differentiation, or a physiological process that has been altered. In some cases the levels of this or other heat shock proteins in the thyroid gland may indicate normal (Ginsberg et al., 2006) or pathological (Medeiros-Neto et al., 1996; Boltze et al., 2003; Baryshev et al., 2004) thyroid functioning. There is even an important relationship between the activity of HSP-90 and the degree of hypophyshary stimulation of the thyroid cells (Ginsberg et al., 2006).

After exposing the rat thyroid gland to radiation, HSP-70 levels also decreased, with a rapid recovery that is probably related to the reparative or cytoprotective effects of this protein (Rajdev and Sharp, 2000). In any case, the HSP-90 and HSP-70 levels accompanying thyroid cells are present under normal physiological conditions and in pathological changes. Thus, human thyroid cell experimental models are optimal for studying how HSP-90 and HSP-70 expression responds to stress from thermal or chemical stimuli in auto-immune diseases of the thyroid gland (Youse et al., 1998).

In spite of the fact that various thyroid pathologies involve increases in HSP-90 and HSP-70 (Medeiros-Neto et al., 1996; Boltze et al., 2003; Baryshev et al., 2004), there is also evidence of situations where conditions such as hyperthermia, aging or illness can diminish the response of heat shock proteins in various tissues (Gutsmann-Conrad et al., 1998; Pardue et al., 2007). This suggests that an increase in heat shock protein synthesis may be necessary in some cellular responses, but not all (Carper et al., 1987). The decrease in the chaperone levels may indicate that the magnitude of heat shock protein responses in various types of cells may affect the survival of these cells after stress (Pardue et al., 2007). Great diversity of vulnerability and response to stress by induction of heat shock proteins has been observed in nervous system tissue studies of different animals (Jorge-Mora et al., 2010; Gutsmann-Conrad et al., 1998).

The similar levels observed for Heat Shock Proteins 90 and 70 indicate a common, non-discriminatory functioning mechanism, as was observed in some cases of continuous and planned therapeutic stimulation of Heat Shock Factor-1 (HSF-1) (Powers and Workman, 2007). This induces the increase in both HSP-90 and 70 chaperones (Kieran et al., 2004). Is it possible that the radiation from a non-thermal mechanism could inhibit (the effects of) HSF1 in some organs or tissues? This hypothesis will require investigation in the future.

However, the two chaperones have different functions in mammals, which are manifest in their different responses to the same physical stimulus (Parcellier et al., 2003; Laneau et al., 2007). Thus, HSP-90 thyroid cells are more sensitive to 3 W

| Power | Before | 0 minutes | 30 minutes | 60 minutes | 90 minutes | 24 hours |
|-------|--------|-----------|------------|------------|------------|----------|
| 0 W   | 36.9±14.10^-2 | 36.9±14.10^-2 | 37.3±20.10^-2 | 37.1±20.10^-2 | 37.1±20.10^-2 | 37.1±20.10^-2 |
| 1.5 W | 37.0±4.10^-2  | 37.5±18.10^-2 | 37.4±13.10^-2 | 37.2±26.10^-2 | 37.3±10.10^-2 | 37.0±14.10^-2 |
| 3 W   | 37.0±13.10^-2 | 37.5±10.10^-2 | 37.6±24.10^-2 | 37.2±25.10^-2 | 37.4±40.10^-2 | 37.1±17.10^-2 |
| 12 W  | 36.8±19.10^-2 | 37.7±12.10^-2 | 37.5±11.10^-2 | 37.5±11.10^-2 | 37.4±15.10^-2 | 37.2±1.10^-2 |
Effects of electromagnetic fields at 2.45 GHz on rat thyroid gland

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Calini, V., Urani, C. and Camatini, M. (2003). Overexpression of HSP70 is induced by ionizing radiation in CHI 101/2 cells and protects from DNA damage. Toxicol. In Vitro 17, 561-566.

Caraglia, M., Marra, M., Mancinelli, F., D’Ambrosio, G., Massa, R., Giordano, A., Budillon, A., Abbruzzese, A. and Bismuto, E. (2005). Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells. J. Cell. Physiol. 204, 539-548.

Carper, S. W., Duffy, J. J. and Gerner, E. W. (1987). Heat shock proteins in thermotolerance and other cellular processes. Cancer Res. 47, 5249-5255.

Chauhan, V., Mariampillai, A., Gajda, G. B., Thansandote, A. and McNamee, J. P. (2006). Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int. J. Radiat. Biol. 82, 347-354.

Cucio, G., Ferrara, M., Moroni, F., D’Inzenz, G., Bertini, M. and De Gennaro, L. (2005). Is the brain influenced by a phone call? An EEG study of resting wakefulness. Neurosci. Res. 53, 265-270.

Dallmann, R., Steinecker, S., von Hörsten, S. and Karl, R. (2006). Stress-induced hyperthermia in the rat: comparison of classical and novel recording methods. Lab. Anim. 40, 136-193.

de Pomerai, D., Daniels, C., David, H., Allan, J., Duke, I., Mutwakil, M., Thomas, D., Sewell, P., Tattersall, J., Jones, D. et al. (2000). Non-thermal heat-shock response to microwaves. Nature 405, 417-418.

Joly, A.-L., Wettstein, G., Mignot, G., Ghiringhelli, F. and Garrido, C. (2010). Pulse modulated 900 MHz heat shock protein in vitro. J. Immunol. 184, 573-579.

Kang, C.-M., Park, K.-P., Cho, Y. and Park, W.-Y. (2003). Non-thermal heat-shock response to microwaves. (Retraction). Nature 440, 437.

Esmeayka, M. A., Seyhan, N. and Ömeroğlu, S. (2010). Pulse modulated 900 MHz radiation induces hypothryroidism and apoptosis in thyroid cells: a light, electron microscopy and immunohistochemical study. Int. J. Radiat. Biol. 86, 1106-1116.

Ginsberg, L., Labeld, T. and Brandley, D. N. (2006). Phosphorylation of heat shock protein-90 by THF in FRTL-5 thyroid cells. Thyroid 16, 737-742.

Gupta, S. C., Sharma, A., Mishra, M., Mishra, R. K. and Chowdhuri, D. K. (2010). Heat shock proteins in toxicology: how close and how far? Life Sci. 86, 377-384.

Greensmith, L., Heydari, A. F., You, S. and Richardson, A. (1998). The expression of heat shock protein 70 decreases with cellular senescence in vitro and in cells derived from young and old human subjects. Exp. Cell Res. 241, 404-413.

Helmbrøeck, K., Zeise, E. and Rensing, L. (2000). Chaperones in cell cycle regulation and mitogenic signal transduction: a review. Cell Signal 12, 341-365.

Jarosz, D. F. and Lindquist, S. (2010). Hsp90 and environmental stress transform the adaptive value of natural genetic variation. Science 330, 1820-1824.

Jin, Y.-B., Lee, H.-J., Seon Lee, J., Pack, J.-K., Kim, N. and Lee, Y.-S. (2011). One-year simultaneous combined exposure of CDMA and WCDMA radiofrequency electromagnetic fields to rats. Int. J. Radiat. Biol. 87, 416-423.

Joly, A.-L., Wettstein, G., Mignot, G., Ghiringhelli, F. and Garrido, C. (2010). Dual role of heat shock proteins as regulators of apoptosis and innate immunity. J. Innate Immun. 2, 238-247.

Jorge-Mora, T., Alvarez-Folgueiras, M., Leiro-Vidal, J. M., Jorge-Barreiro, F. J., Ares-Pena, F. J. and López-Martin, E. (1992). Exposure to 2.45 GHz microwave radiation provokes cerebral changes in induction of HSP-90 in heat shock protein in rat. PIER 100, 351-379.

Josifov, T. A., Mura, M., Zuccato, L., Morkos, S. and Boccardo, N. (2007). Analysis of heat shock protein 70 in human brain tumors. J. Cancer Res. Treat. 41, 78-84.

Kang, K.-I., Bouchou, L., Fortin, D. and Mutel, J.-P. (2011). Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Life Sci. 32, 1822-1829.

Karasek, M. and Woldanska-Okonska, M. (2004). Electromagnetic fields and natural immunity. Immunopathol. Pharmacol. 62, 398-408.

Kieran, D., Kalmar, B., Dick, J. R., Riddoch-Contrares, J., Burnstock, G. and Greensmith, L. (2004). Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat. Med. 10, 402-405.

Koyu, A., Cesur, G., Oğuzer, F., Akdogan, M., Mollaoglu, H. and Ozden, S. (2005). Effects of 900 MHz electromagnetic field on TSH and thyroid hormones in rats. Toxicol. Lett. 157, 257-262.

Kregel, K. C. (2002). Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol. 92, 2177-2186.

References

Alfieri, R. R., Bonelli, M. A., Pedrazzi, G., Desenzani, S., Ghiliani, M., Fumara, C., Ghibelli, L., Borghetti, A. F. and Petronini, P. G. (2006). Increased levels of inducible HSP70 in cells exposed to electromagnetic fields. Radiat. Res. 165, 95-104.

Baryshev, M., Sargsyan, E., Wallin, G., Lejnieks, A., Furudate, S., Hishinuma, A. and Murtchian, S. (2004). Unfolded protein response is involved in the pathology of human congenital hypothyroid goiter and rat non-goitrogen congenital hypothyroidism. J. Med. Endocrinol. 32, 903-920.

Bergamaschi, A., Magrini, A., Ales, G., Coppeta, L. and Somma, G. (2004). Are thyroid dysfunctions related to stress or microwave exposure (900 MHz)? Int. J. Immunopathol. Pharmacol. 17 Suppl, 31-36.

Boltze, C., Schneiker-Schroth, R., Roesser, A., Quednow, C. and Hoang-Vu, C. (2003). Function of HSP90 and p23 in the telomerase complex of thyroid tumors. Pathol. Res. Pract. 199, 573-579.

Calini, V., Urani, C. and Camatini, M. (2003). Overexpression of HSP70 is induced by ionizing radiation in CHI 101/2 cells and protects from DNA damage. Toxicol. In Vitro 17, 561-566.

Caraglia, M., Marra, M., Mancinelli, F., D’Ambrosio, G., Massa, R., Giordano, A., Budillon, A., Abbruzzese, A. and Bismuto, E. (2005). Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells. J. Cell. Physiol. 204, 539-548.

Carper, S. W., Duffy, J. J. and Gerner, E. W. (1987). Heat shock proteins in thermotolerance and other cellular processes. Cancer Res. 47, 5249-5255.

Chauhan, V., Mariampillai, A., Gajda, G. B., Thansandote, A. and McNamee, J. P. (2006). Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int. J. Radiat. Biol. 82, 347-354.
Effects of electromagnetic fields at 2.45 GHz on rat thyroid gland

Lanneau, D., de Thonel, A., Maurel, S., Didelot, C. and Garrido, C. (2007). Apoptotic versus cell differentiation: role of heat shock proteins HSP90, HSP70 and HSP27. Prion 1, 53-60.

Lee, S., Johnson, D., Dunbar, K., Dong, H., Ge, X., Kim, Y. C., Wing, C., Jayathilaka, N., Emmauel, N., Zhou, C. Q. et al. (2005). 2.45 GHz radiofrequency fields alter gene expression in cultured human cells. FEBS Lett. 579, 4829-4836.

Lope, V., Pérez-Gómez, B., Aragonés, N., López-Abente, G., Gustavsson, P., Flererus, B., Dosemeci, M., Silva, A. and Pollán, M. (2006). Occupational exposure to ionizing radiation and electromagnetic fields in relation to the risk of thyroid cancer in Sweden. Scand J. Work Environ. Health 32, 276-284.

López-Martín, E., Relova-Quintela, J. L., Gallego-Gómez, R., Peleteiro-López, V., Peñafiel, J. F. and Ares-Pena, F. J. (2008). GSM radiation triggers seizures and increases cerebral c-Fos positivity in rats pretreated with subconvulsive doses of picrotoxin. Neurosci. Lett. 398, 139-144.

López-Martín, E., Bregains, J., Relova-Quintela, J. L., Cadarso-Suárez, C., Jorge-Barreiro, F. J. and Ares-Pena, F. J. (2009). The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. J. Neurosci. Res. 87, 1484-1499.

Malaya, R., Sahu, E. W., Bi, C., Straube, W. L., LaRegina, M., Pickard, W. F. and Rolfi Reit, J. L. (1998). DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. Radiat. Res. 149, 637-645.

Medeiros-Neto, G., Kim, P. S., Yoo, S. E., Vono, J., Targovnik, H. M., Camargo, R., Hossain, S. A. and Arvan, P. (1996). Congenital hypothyroid goiter with deficient thyroglobulin. Identification of an endoplasmic reticulum storage disease with induction of molecular chaperones. J. Clin. Invest. 98, 2838-2844.

Milham, S. and Morgan, L. L. (2008). A new electromagnetic exposure metric: high frequency voltage transients associated with increased cancer incidence in teachers in a California school. Am. J. Ind. Med. 57, 579-586.

Morimoto, R. I. (1993). Cells in stress: transcriptional activation of heat shock genes. Science 259, 1409-1410.

Morimoto, R. I., Sarge, K. D. and Abravaya, K. (1992). Transcriptional regulation of heat shock genes. A paradigm for inducible genomic responses. J. Biol. Chem. 267, 21987-21990.

Paggi, A., Di Prima, M. A., Paparo, B. S., Pellegrino, C., Faralli, A. R., Sinopoli, M. T. and Leri, O. (1993). Anti 70 kDa heat shock protein antibodies in sera of patients with autoimmune and non-autoimmune thyroid diseases. Endocr. Res. 21, 555-567.

Parecellier, A., Gurbuxani, S., Schmitt, E., Solary, E. and Garrido, C. (2003). Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochem. Biophys. Res. Commun. 304, 505-512.

Pardue, S., Wang, S., Miller, M. M. and Morrison-Bogordar, M. (2007). Elevated levels of inducible heat shock 70 proteins in human brain. Neurobiol. Aging 28, 314-324.

Paulraj, R. and Behari, J. (2006). Protein kinase C activity in developing rat brain cells exposed to 2.45 GHz radiation. Electromagn. Biol. Med. 25, 61-70.

Perez, F. P., Zhou, X., Morisaki, J. and Jurvich, D. (2008). Electromagnetic field therapy delays cellular senescence and death by enhancement of the heat shock response. Exp. Gerontol. 43, 307-316.

Powers, M. V. and Workman, P. (2007). Inhibitors of the heat shock response: biology and pharmacology. FEBS Lett. 581, 3758-3769.

Rajdev, S. and Sharp, F. R. (2000). Stress proteins as molecular markers of neurotoxicity. Toxcol. Pathol. 28, 105-112.

Rajdev, V., Matavulj, M., Gledic, D. and Lazetic, B. (2003). Evaluation of rat thyroid gland morphophysiological status after three months exposure to 50 Hz electromagnetic field. Tissue Cell 35, 223-231.

Rajdev, V., Matavulj, M. and Johansson, O. (2005). Histological characteristics of cutaneous and thyroid mast cell populations in male rats exposed to power-frequency electromagnetic fields. Int. J. Radiat. Biol. 81, 491-499.

Rajdev, V., Matavulj, M. and Johansson, O. (2006). Light and electron microscopic study of the thyroid gland in rats exposed to power-frequency electromagnetic fields. J. Exp. Biol. 209, 3322-3328.

Ramañía, V. and Bück, M. (2004). Time-dependent expression of heat shock proteins 70 and 90 in tissues of the anoxic western painted turtle. J. Exp. Biol. 207, 3775-3784.

Regel, S. J. and Achermann, P. (2011). Cognitive performance measures in bioelectromagnetic research—critical evaluation and recommendations. Environ. Health 10, 10.

Samadi, A., Lee, P., Mekerji, R., O’Donnell, G., Tong, X., Timmermann, B. N. and Cohen, M. S. (2009). A novel HSP90 modulator with selective activity against thyroid cancers in vitro. Surgery 146, 1196-1207.

Santin, A. P. and Furlanetto, T. W. (2011). Role of estrogen in thyroid function and growth regulation. J. Thyroid Res. 2011, 875125.

Schmidt, E., Gehrmann, M., Brunet, M., Multhoff, G. and Garrido, C. (2007). Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. J. Leukoc. Biol. 81, 15-27.

Tian, F., Nakahara, T., Wake, K., Taki, M. and Miyakoshi, J. (2002). Exposure to 2.45 GHz electromagnetic field induces hsp70 at a high SAR of more than 20 W/kg but not at 5 W/kg in human glioma MO54 cells. Int. J. Radiat. Biol. 78, 433-440.

Trautinger, F., Kindás-Mügge, I., Knobler, R. M. and Höningmann, H. (1996). Stress proteins in the cellular response to ultraviolet radiation. J. Photochem. Photobiol. B 35, 141-148.

Valbonesi, P., Franzellitti, S., Piano, A., Contiu, A., Biondi, C. and Fabбри, E. (2008). Evaluation of HSP70 expression and DNA damage in cells of a human trophoblast cell line exposed to 1.8 GHz amplitude-modulated radiofrequency fields. Radiat. Res. 169, 270-279.

Wakefield, R. (2004). The cancer epidemiology of radiation. Oncogene 23, 6404-6428.

Wallin, G., Brännegård, M., Lindberg, S. S., McGuire, J. and Tørring, O. (2007). Expression of the thyroid hormone receptor, the oncopgenes c-myc and H-ras, and the 90 kD heat shock protein in normal, hyperplastic, and neoplastic human thyroid tissue. Thyroid 2, 307-313.

Wang, J., Koyama, S., Komatsubara, Y., Suzuki, Y., Wake, K., Watanabe, S., Taki, M., Ueno, S. and Nagawa, H. (2010). Short-term exposure to a 1439-MHz TDMA signal exerts no estrogenic effect in rats. Bioelectromagnetics 31, 573-575.

Yang, S. H., Liu, R., Wen, Y., Perez, E., Cutright, J., Brun-sinkenagel, A. M., Singh, M., Day, A. L. and Simpkins, J. W. (2005). Neuroendocrine mechanism for tolerance to cerebral ischemia-reperfusion injury in male rats. J. Neurobiol. 62, 341-351.

Youde, S. J., Mower, J., Moore, D. P. and Parkes, A. B. (1998). Stress protein expression in primary and immortalized cultures of human thyroid cells: a model system for the study of stress proteins in the pathogenesis of autoimmune thyroid disease. Cell Stress Chaperones 3, 89-93.