Effects of the exposure of 2.14GHz W-CDMA radiofrequency electromagnetic fields to rats on body temperature increase and heat shock proteins expression

Akira Ushiyama[*1], Shin Ohtani[2], Machiko Maeda3, Kenji Hattori[4], Naoki Kunugita[5], Jianqing Wang[6], and Kazuyuki Ishii[7]*

[1]Department of Environmental Health, National Institute of Public Health
2-3-6 Minami, Wako, Saitama, 351-0197, Japan,
tel +81-48-458-6257, fax +81-458-6270, e-mail ushiyama@niph.go.jp

[2]Department of Hygienic Chemistry, Meiji Pharmaceutical University
2-522-1 Noshio, Kiyose,Tokyo 204-8588 Japan
e-mail ohtani@niph.go.jp

[3]As 2 above, but e-mail: machikom@niph.go.jp

[4]As 2 above, but e-mail:khattori@my-pharm.ac.jp

[5]As 1 above, but e-mail: kunugita@niph.go.jp

[6]Department of Electrical and Electronic Engineering, Nagoya Institute of Technology
Gokiso-cho, Showa-ku, Nagoya, Aichi, 466-8555 Japan
e-mail : wang@nitech.ac.jp

[7] As 2 above, but e-mail: ishii@my-pharm.ac.jp

Abstract

The radio frequency-electromagnetic field (RF-EMF) exposure and heat shock protein (Hsp) expression has been discussed, but there is no data on the relationship between thermal increase by RF-EMF and Hsp expression. Rats were exposed to RF-EMFs [2.14 GHz, wideband-code division multiple access (W-CDMA) signals] for 2 h/day for 3 days or 1 h/day for 10 days with a whole body average-specific absorption rate (WBA-SAR) of 4 W/kg or 0.4 W/kg, respectively. It appeared the intraperitoneal temperature was increased during RF-EMFs exposure and the *Hsp27* and *Hsp110* genes were significantly upregulated in the cerebral cortex and cerebellum at 4 W/kg, but protein upregulation was not detected. In contrast, there was no significant change in any of the genes at 0.4 W/kg. These results indicated that the thermal increase of the body by RF-EMF exposure plays critical role on Hsp expression.

1. Introduction

Under the high power of RF-EMFs exposure, RF-EMF may act as a stress inducer and mediate stress responses through stress proteins. These responses may rely on the synthesis of heat shock proteins (Hsps), although the mechanism of induction has not yet elucidated. Most reports have demonstrated changes in expression levels of Hsps as a response to EMF exposure. Fritze et al. [1] found that RF-EMF exposure with a specific absorption rate at 7.5W/kg slightly induced Hsp70 mRNA on the cerebellum and hippocampus after exposing rats to 900MHz pulse waves. Yang et al. [2] also reported that 2.45 GHz RF-EMF exposure at SAR 6W/kg to rats resulted the increases of *Hsp27* and *Hsp70* for both mRNA and protein levels. These results might be reasonable because the high-power RF-EMFs are absorbed as heat energy into the body. To date, many studies have been conducted as described above, but they have focused on changes in the Hsp family at the molecular level and no data are available on the relationship between thermal changes and Hsps expressions. In this study, we therefore investigated whether RF-EMF exposure to 4 W/kg WBA-SAR increased thermal stress *in vivo*, by monitoring thermal changes during exposure and analyze the Hsps expression.

978-1-4673-5225-3/14/$31.00 ©2014 IEEE

2. Materials and Methods

Animals

Male Sprague-Dawley rats (6 weeks old) were purchased from Japan SLC. These rats were kept in acrylic cages and housed in an air-conditioned specific pathogen-free animal room on a 12 h-light/12-h dark cycle. The temperature and relative humidity were maintained at 23°C ± 1°C and approximately 55%, respectively.

RF-EMF exposure

The reverberation chamber system [3] was used for exposure. It can emit high-power RF-EMFs uniformly, and the WBA-SAR was calculated by computer simulation. A 2.14-GHz W-CDMA signal and 4-W/kg and 0.4-W/kg WBA-SARs were used in this study. The exposure terms were 3 consecutive (2 h/day) or 10 consecutive (1 h/day) days. To expose the accurate SAR, an acrylic cage containing 4 rats per cage was set in the center of the chamber.

Intraperitoneal temperature monitoring and Sample collection

Prior to the exposure, a small thermo data logger (Thermochron SL type; KN Laboratories Inc. Japan) was implanted into the abdominal cavity by surgical procedure. During exposure period, the intraperitoneal temperature was recorded at every 1 minute. At the end of all of the exposure experiments, rats were euthanized and data logger was excised for collecting data. The right hemisphere of the brain was also dissected and removed from the rats, soaked in RNA later(Qiagen, Valencia, CA, USA), and stored at −80°C until RNA extraction. The cerebral cortex and cerebellum were removed from the left hemisphere of the brain with disposable razors and homogenized with lysis buffer. After ultracentrifugation, the soluble fractions were stored at −80°C until western blotting.

Gene expression analysis

Total RNA was extracted from sham-exposed (n = 4 or 6), 0.4 W/kg-exposed (n = 6), and 4 W/kg-exposed (n = 4 or 6) rats with RNeasy Mini Kits (Qiagen). The resulting RNA was reverse transcribed with High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster, CA, USA). Quantitative PCR was performed with Fast SYBR Green Master Mix (Applied Biosystems). All of the primers were designed with Primer3 within the amplified fragment length of 150 base pairs, which was purchased from Exigen Tokyo, Japan. The PCR was performed at 95°C and 58°C for 45 cycles in a Stratagene Mx3000P QPCR system (Agilent Technologies, Palo Alto, CA, USA). All of the samples were assayed in triplicates. The relative levels of expression of each mRNA were determined according to the comparative cycle threshold method with equation $2^{-\Delta\Delta CT}$.

Western blotting

Proteins of the cerebral cortex and cerebellum were extracted in hypotonic lysis buffer. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. After blocking, the membranes were incubated with primary antibodies against β-actin (Cell Signaling Technology, Danvers, MA, USA), Hsp27 (GeneTex, San Antonio, TX, USA), and Hsp110 (Novus Biologicals, Littleton, CO, USA) and then incubated with horseradish peroxidase-conjugated anti-IgG secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). The protein bands were visualized with Chemi-Lumi One L western blotting substrate (Nacalai Tesque, Kyoto, Japan), and the bands intensities were measured with Image Lab software (Bio-Rad, Hercules, CA, USA).

Statistics

Comparisons of the relative expression levels of mRNA were examined with unpaired Student's t-tests or Welch's t-tests between the sham and exposed groups. The levels of significance were set at $P < 0.05$ and $P < 0.01$. The statistical analyses were performed with Microsoft Excel.

3. Results and Discussion

During the RF-EMF exposure at SAR 4W/kg, the intraperitoneal temperature rise about 1.5 ^{o}C compared to pre-exposure condition. In addition, it kept equilibrium in the temperature for the exposure of 6 hours. When the exposure ended, the temperature fell immediately to basal range. The gene expression levels of the Hsp family (*Hsp27, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp110*) and the Hsf family (*Hsf1, Hsf2, and Hsf4*) were analyzed with real-time PCR assays in the cerebral cortex and the cerebellum. At 4 W/kg WBA-SAR for both 3 and 10 consecutive days, the *Hsp27* and *Hsp110* genes were significantly upregulated in the outside part of the cerebral cortex and cerebellum, but there were no changes in the whole cerebral cortex and cerebellum, which also included the inside parts. This data suggested

that Hsp27 and Hsp110 may be more affected by RF-EMF exposure at 4 W/kg WBA SAR in the outside of the brain than in the inside of the brain. These might be due to thermal effect mentioned above. However, we could not detect significant changes in Hsp27 and Hsp110 protein levels. In contrast there were no significant changes in the Hsp27 and Hsp110 genes or any of the other genes at 0.4 W/kg WBA-SAR. Therefore, there were no (or little) thermal effects on Hsp and Hsf gene expression in the cerebral cortex and cerebellum at 0.4-W/kg WBA-SAR. These findings suggested that the threshold for the upregulation of Hsp27 and Hsp110 gene expression in rat brains exposed to RF-EMFs (2.14 GHz, W-CDMA signal) was between 0.4 W/kg and 4 W/kg WBA-SAR.

4. References

1. 1: Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, Kiessling M, Hossmann KA. Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. *Neuroscience*. 1997; 81(3):627-39.

2. Yang XS, He GL, Hao YT, Xiao Y, Chen CH, Zhang GB, Yu ZP. Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. *Brain Res Bull*. 2012; 88(4):371-8.

3. Chakarothai J, Wang J, Fujiwara O, Wake K, Watanabe S. Dosimetry of a reverberation chamber for whole-body exposure of small animal, *IEEE Trans. Microwave Theory Tech.* 2013; 61(9):.3435-45.