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LETTER TO THE EDITOR

Effects of exposure to 1.8 GHz radiofrequency field on the expression of Hsps and phosphorylation of MAPKs in human lens epithelial cells

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Dear Editor,

The growing public concerns about the possible health effects of exposure to radio frequency (RF) fields from mobile telephones have arisen in many countries because of the increased use of mobile telecommunication devices. This in turn has led to an increase in cognate epidemiological and experimental investigations. However, the results of these studies are conflicting. Some have implicated an increased health risk [1], but most studies have shown no effects [2]. The lens epithelial cells (LECs) are a single layer of cuboidal cells at the anterior surface of the lens, which retain the proliferative capacity and are important for maintaining the metabolic homeostasis and transparency of the lens. Damage to LECs has been found to be associated with cataractogenesis. To investigate whether exposure to 1.8 GHz RF can influence the cellular physiology of the LECs, the expression of heat shock proteins (Hsps) and the activation of mitogen-activated protein kinases (MAPKs) in human LECs after exposure to the 1.8 GHz RF of a global system for mobile communications (GSM) were evaluated. The average specific absorption rate (SAR) level of 2 W/kg is the safety limit for mobile phone microwave radiation emission as defined by the ICNIRP (International Commission on Non-Ionizing Radiation Protection). Furthermore, as per the guidelines of the ICNIRP in 1998, the available experimental evidence indicated that the threshold for irreversible effects in even the most sensitive tissues was greater than 4 W/kg, which is recommended as the occupational whole-body exposure restriction with a traditional safety factor of ten, which translates to 0.4 W/kg. On the other hand, we intended to obtain obvious bio-effects of microwave radiation so as to investigate the potential mechanism of microwave-induced cataract. Therefore, we chose the SARs of 1, 2, 3, and 4 W/kg in our study. During the entire course of exposure, the temperature never fluctuated over the range of 37±0.108 ºC; all data and experimental settings were stored by the computer every 10 s. The temperature difference between sham and exposure never exceeded 0.108 ºC.

Hsps are a family of stress-activated proteins that participate in protein folding, repair, and degradation, which characterize the cellular responses to various types of stresses, such as changes in pH, heavy metal, and sudden temperature increases [3]. Hsps are classified into four major families according to their molecular weights: Hsp90, Hsp70, and Hsp60, and the small Hsps, such as Hsp10, Hsp27, αA-crystallin, and αB-crystallin. A main function of these proteins is to assist in protein folding. Hsps also help to prevent apoptosis after a stress stimulus, allowing time for the repair mechanisms to act. We evaluated Hsp expression by western blot analysis immediately after exposure to RF fields (SAR: 1, 2, 3, 4 W/kg) for 2 h. Although a significant change in Hsp27 and Hsp70 was observed in RF-exposed cells (SAR: 2, 3, 4 W/kg) compared with sham-exposed cells, no significant difference was observed for Hsp90 (Figure 1A and 1B). It has been reported that exposure of human reconstructed epidermis (hRE) to 900 MHz RF at 2 W/kg for 48 h induced a slight but significant increase in Hsp70 expression [4]. Kwee *et al*. [5] have reported that the expression of Hsp70, but not Hsp27, was induced when transformed human epithelial amnion cells were exposed to a GSM signal of 960 MHz at

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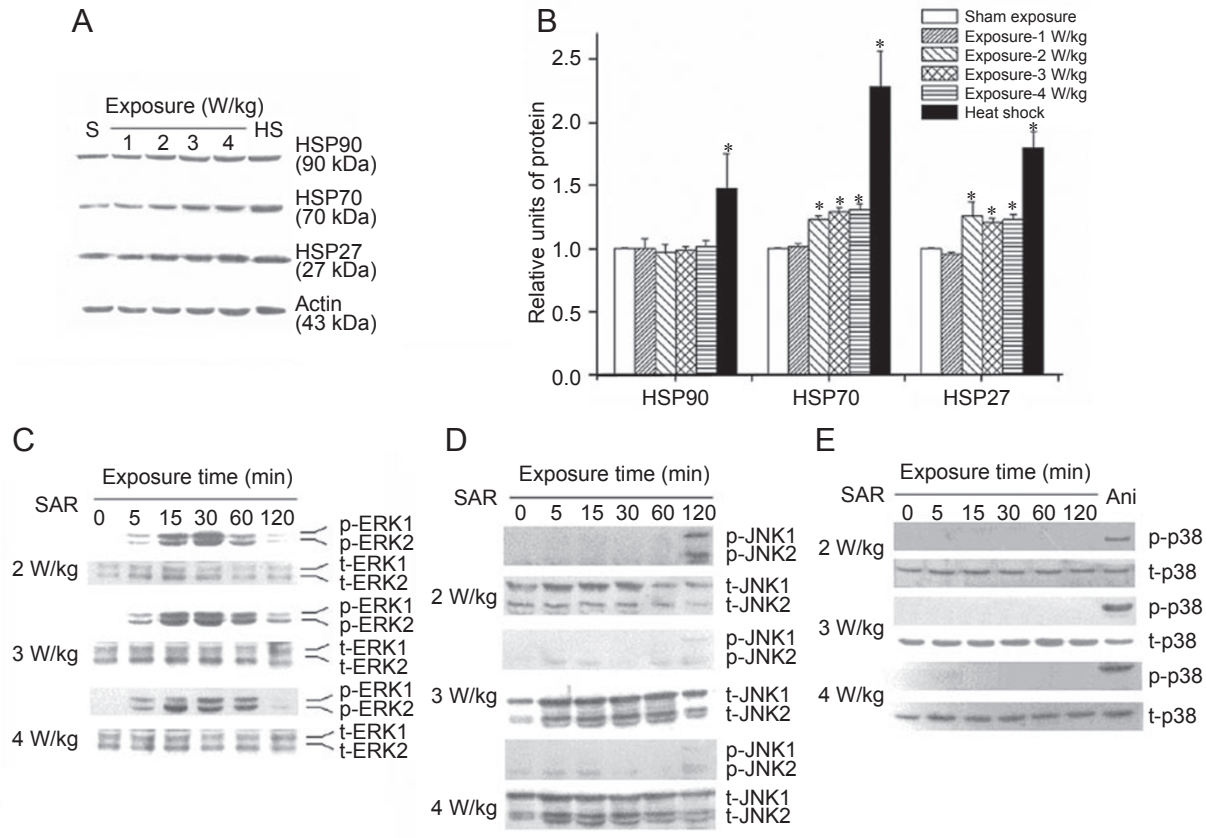

Figure 1 (A) Western blot analysis of Hsp90, Hsp70, and Hsp27 in human LECs immediately after exposure to RF field (SAR: 1, 2, 3, 4 W/kg) for 2 h. **(B)** Data of Hsp protein levels are representative of at least three independent experiments. The intensity of bands was quantitated by densitometry. Each data point represents the mean±SD (n = 3). *$P < 0.05$, compared with sham. HS: heat shock (positive control). **(C)** Effect of RF field on the phosphorylation of ERK1/2 in human LECs. The cell culture medium was not changed prior to irradiation. **(D)** Effect of RF field on the phosphorylation of JNK1/2 in human LECs. **(E)** Effect of RF field on the phosphorylation of p38 in human LECs. As a positive control, cells were stimulated with anisomycin (ANISO, 10 μg/ml) for 30 min. Ani: Anisomycin. In **(C-E)**, cells were exposed to RF field at SARs of 2, 3, and 4 W/kg for 5, 15, 30, 60, and 120 min. The experiments were repeated three times.

a SAR of 0.0021 W/kg for 20 min. However, Leszczynski *et al*. [6] showed that non-thermal exposure of cultures of a human endothelial cell line to 900 MHz GSM microwave radiation for 1 h did cause a transient change in Hsp27. Our present study showed that 1.8 GHz RF exposure could induce the up-regulation of Hsp27 and Hsp70 when SAR was ≥ 2W/kg. This is in contrast to other reports that showed that intermittent (5 min on, 10 min off) exposure to 1.9 GHz RF field at an average SAR of 1 and 10 W/kg, respectively, for 6 h did not affect the mRNA expression of Hsp27 and Hsp70 in HL-60 and MM6 cells [7], and that 1.9 GHz RF field had no effect on the expression of Hsp27 or Hsp70 in human glioma cells at SARs of 1, 2, and 10 W/kg for 1 and 2 h [8]. These conflicting findings may have resulted from the different cell lines and different exposure patterns used in the experiments.

The MAPKs comprise three major types, extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38, which are involved in the signal transduction pathways in all eukaryotes and mediate the effects of various stimuli to regulate essentially all stimulated processes, including proliferation, differentiation, metabolism, and the stress response [9]. It has been proposed that activation of JNKs and p38 MAP kinases contributes to cell death, whereas activation of ERKs contributes to protection against cell injury in multiple organs [10]. There is evidence that induction of the MAPK pathway plays significant roles in the activation of specific Hsps [11]. We thus examined the activation of MAPKs after exposure to RF at SARs of 2, 3, and 4 W/kg for 0, 5, 15, 30, 60, and 120 min. The results showed that ERK1/2 was markedly activated as early as 5 min after RF exposure; the activation peaked at 30 min and lasted up to 2 h after exposure (Figure 1C). Phosphorylation of JNK1/2 was detected at

2 h after exposure (Figure 1D), while p38 activation was not detected (Figure 1E). Recently, it was reported that ERK was activated within 5 min of the radiation, with the peak activity occurring within 10-15 min, whereas no phosphorylation of JNKs or p38 was detected within a short time of exposure [12]. Our results with human LECs are consistent with that study. These results indicate that mobile phone radiation can induce an immediate effect in the cytoplasm that activates ERK signaling so as to further induce transcription of a variety of genes [13], and that long exposure can activate JNK. However, we did not find the activation of p38 after RF exposure. The appearance of p-ERK1/2 and p-JNK1/2, together with Hsp up-regulation, suggests that non-thermal RF exposure can induce the stress response in human LECs.

Our results suggest that exposure to RF of wireless communications can induce expression of Hsp27 and Hsp70 and the activation of ERK1/2 and JNK1/2 in human LECs. The induction of Hsp27 and Hsp70, by a non-thermal stress, together with the activation of signal transduction pathways, provides reliable and sensitive biomarkers that could serve as the basis for improved mobile phone safety guidelines.

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