EFFECT OF GSM MOBILE PHONE RADIATION ON BLOOD-BRAIN BARRIER

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ABSTRACT

Some animal studies have suggested that mobile phone radiation may cause increase in blood-brain barrier permeability. We have hypothesized (Leszczynski et al. Differentiation, 70, 2002, in press) that the mobile phone radiation-induced increased expression and phosphorylation (activity) of stress protein hsp27 might be the molecular mechanism regulating blood-brain barrier permeability and, possibly, cell apoptosis. Here we present evidence suggesting that mobile phone radiation indeed affects hsp27-dependent cytoplasmic distribution of F-actin and stability of stress fibers. This observation supports our hypothesis that mobile phone radiation-induced changes in hsp27 expression/activity might eventually lead to increase in the permeability of blood-brain barrier.

BACKGROUND

The question whether microwave radiation, that is emitted by mobile phones (radio-frequency modulated electromagnetic fields: RF-EMF), might exert any detrimental health effects remains unanswered. Several recently conducted reviews of the to-date published research have concluded that there is significant and credible scientific evidence to the fact that RF-EMF induces biological effects [1,2,3,4]. However, it still remains to be determined whether these biological responses could cause health hazard.

The possibility of the induction of cellular stress response by the non-thermal levels of mobile phone radiation has been shown just recently. In vivo, Daniells et al. [5] and de Pomerai et al. [6] have shown that overnight irradiation of nematode worms with RF-EMF (750MHz) at SAR of 0.001W/kg causes increase in expression of heat shock protein. Fritze et al. [7], using rat model, have shown increase in expression of stress protein hsp70 in brains of animals exposed for 4 hours to RF-EMF (890-915MHz) at SAR of 1.5W/kg. In vitro, Kwee et al. [8] have shown induction of stress protein hsp70, but not hsp27, in transformed human epithelial amnion cells exposed for 20 min. to RF-EMF (960MHz) at SAR of 0.0021W/kg. Thus, because of the known broad spectrum of physiological processes that are regulated by stress proteins [9], it is possible to suggest that mobile phone radiation-induced activation of cellular stress response might affect variety of physiological processes, among them brain tumor development and blood-brain barrier permeability. Having this in mind, French et al. [10] have put forward hypothesis suggesting that repeated exposures of cells to mobile phone radiation over a long period of time might affect tumor development due to the hypothesized chronic up-regulation of the expression levels of cellular stress proteins. However, occurrence of such chronic stimulatory effect on the expression of stress proteins induced by mobile phone radiation, as suggested by French et al. [10], still remains to be experimentally demonstrated.

PREVIOUS STUDY

In our earlier study [11] we have demonstrated that the 1-hour non-thermal exposure of human endothelial cell line EA.hy926 to SAR of 2W/kg (900MHz GSM signal) leads, among others, to: (i) changes in phosphorylation status of a large number of proteins, (ii) among them, transient increase in phosphorylation of hsp27 stress response protein, which was prevented by SB203580, a specific inhibitor of p38 mitogen-activated protein kinase (p38MAPK), (iii) transient changes in protein expression levels of hsp27 and p38MAPK.

Over-expression and phosphorylation of hsp27 has been shown to regulate polymerization of F-actin and formation and stability of stress fibers. This, when occurring in endothelial cells lining brain's capillary blood vessels, might be of importance for the functioning of blood-brain barrier. Stabilization of stress fibers and cytoplasmic distribution of F-actin was shown to cause: (i) cell shrinkage, that might lead to opening of spaces between cells, (ii) increase in the permeability and pinocytosis of endothelial monolayer, (iii) increase in formation of the so called “apoptosis-unrelated” blebs on the surface of endothelial cells, which eventually might obstruct blood flow through capillary blood vessels, (iv) stronger responsiveness of endothelial cells to estrogen and, when stimulated by this hormone, to secrete larger than normally amounts of basic fibroblast growth factor (bFGF) which might, in endocrine manner, stimulate de-

differentiation and proliferation of endothelial cells and possibly led to the associated with cell's proliferative state - cell shrinkage and unveiling of basal membrane.

The possibility of the effect of RF-EMF exposure on blood-brain barrier permeability has been suggested earlier by in vivo [12] and in vitro [13] studies. However, there are also reports where authors claim that the non-thermal levels of RF-EMF radiation do not affect blood-brain barrier permeability [14,15]. The no-effect, which is claimed by Fritze et al. [14], is not so straight forward. The authors have observed stress response and increased permeability of the blood-brain barrier immediately after the end of irradiation. This effect was, however short lasting. Therefore, it remains unclear what would be the blood-brain barrier response to the repeated exposures to mobile phone radiation because the effect of repeated exposures was not examined. The increased blood-brain barrier permeability due to increase of pinocytosis was suggested by Neubauer et al. [16] who have demonstrated increase in pinocytosis of cerebral cortex capillaries that were exposed to 2.45 GHz microwave radiation. Finally, the recently reported study by Töre et al. [17] has shown that 2 hour exposure of rats to RF-EMF (900MHz) at SAR of 2W/kg (averaged over the brain) causes increase in the permeability of blood-brain barrier. The molecular mechanism and the cellular signaling pathways involved in the induction of blood-brain barrier permeability are still unknown.

Activated (phosphorylated) hsp27 has been shown to inhibit apoptosis by forming complex with the apoptosome (complex of Apaf-1 protein, pro-caspase-9 and cytochrome *c*), or some of its components, and preventing proteolytic activation of pro-caspase-9 into active form of caspase-9 [18,19]. This, in turn, prevents activation of pro-caspase-3 which is activated by caspase-9. Thus, induction of the increased expression and phosphorylation of hsp27 by the RF-EMF exposure might lead to inhibition of the apoptotic pathway that involves apoptosome and caspase-3. This event, when occurring in RF-EMF exposed brain cells that underwent either spontaneous or external factor-induced transformation/damage, could support survival of the transformed/damaged cells.

HYPOTHESIS

Based on the known cellular role of over-expressed/phosphorylated hsp27 we have proposed a hypothesis [11] that: the activation (phosphorylation) of hsp27 by mobile phone radiation might be the molecular mechanism (i) regulating increase in blood-brain barrier permeability, which would explain, observed in some animal experiments, increase in blood-brain barrier permeability, and (ii) regulating apoptosis through interference with the cytochrome c/caspase-9/caspase-3 pathway (Figure 1).

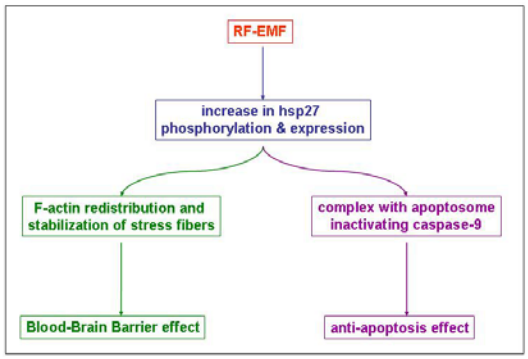

Fig. 1. Hypothetical flow of events that might occur in cells in response to mobile phone radiation.

OBJECTIVE

The present study was undertaken to determine whether physiological responses of endothelial cells, which are associated with the hsp27 expression and phosphorylation and might affect permeability of blood-brain barrier (stability of stress fibers, cell size/shape), occur in the mobile phone radiation exposed cultures of human endothelial cell line EA.hy926.

MATERIAL AND METHODS

Human endothelial cell line EA.hy926 cells, grown on microscope cover slides, were exposed for 1h to 900MHz GSM signal at an average SAR of 2W/kg (range 1.8 – 2.5 W/kg). Temperature of cell cultures remained throughout

irradiation period at 37±0.3°C thus the effects reported here are of non-thermal nature. Cells on cover slides were fixed either immediately or 1h after the end of irradiation. The expression of hsp27 was determined by indirect immunohistochemistry in order to confirm that the cells respond to irradiation in the same way as in the previous study [11]. The appearance of cells (size, shape) and cytoplasmic pattern of F-actin distribution (stabilization of stress fibers) was determined by staining of the cells with fluorescent-dye (AlexaFluor) labeled phalloidin.

RESULTS AND DISCUSSION

As expected, 1h exposure of cells to mobile phone radiation increased expression of hsp27. However, in order to increase hsp27 expression by heat shock was required 3h incubation of cells at 43°C (1h exposure had no effect). This observation, together with the measurements showing that temperature of medium was throughout RF-EMF exposure period at 37±0.3°C, suggest that the observed here effects are of non-thermal nature.

The stability of stress fibers, as determined by the pattern of staining with phalloidin-AlexaFluor, increased after 1h irradiation and did not decline during the 1h of post-irradiation incubation. Induction of the stability of stress fibers caused cells to shrink. In cells expressing high levels of hsp27, the cell edges were brightly stained with phalloidin-AlexaFluor, what indicates re-localization of F-actin to cell ruffles. These cells rounded-up and cells contacted in-between only through thin pseudopods. In cells expressing lower levels of hsp27, network of stress fibers was seen throughout the cytoplasm but not in the ruffles.

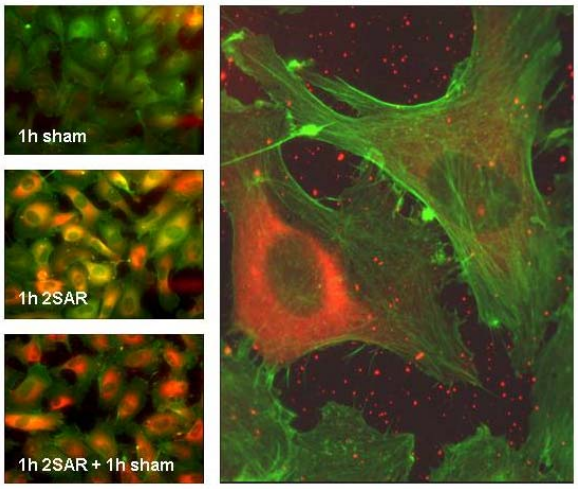

Fig. 2. Expression pattern of F-actin in EA.hy926 cells detected using phalloidin-AlexaFluor staining (green fluorescence) and hsp27 using indirect immunofluorescence (red color). Left panel: cells exposed for 1h to sham, cells exposed for 1h at 2W/kg (2SAR), and cells exposed for 1h at 2W/kg followed by 1h exposure to sham. Right panel: cell expressing high level of hsp27 has F-actin in cell ruffles whereas cell expressing low level of hsp27 has F-actin in form of stress fibers distributed throughout cytoplasm (notice difference in stress fiber density over the nuclear region in both cells).

The observed here, hsp27-related changes in cytoplasmic distribution of F-actin are apparently outcome of two phenomena: hsp27 over-expression and hsp27 phosphorylation. These observed changes support the hypothesis that the hsp27/p38MAPK stress signaling pathway might be the molecular mechanism regulating mobile phone radiation-induced permeability of blood-brain barrier.

CONCLUSIONS

The proposed above intra-cellular mechanism for the mobile phone radiation-increased permeability of the blood-brain barrier is a hypothesis but as such it is reasonably supported by the evidence concerning both effects of microwaves on stress response and effects of hsp27 (increased expression and activity) on cell physiology. Furthermore, it appears that the physiological changes caused by hsp27 phosphorylation indeed take place in endothelial cells (stress fibers' expression, cell size/shape changes). These events, when occurring repeatedly (on daily basis) over the long period of time (years) might become health hazard because of the possible accumulation of brain tissue damage.

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REFERENCES

[1] Jokela K, Leszczynski D, Paile W, Salomaa S, Puranen L, Hyysalo P. (1999). Radiation safety of handheld mobile phones and base stations. STUK-A161 Report, OY Edita Ab Helsinki, Finland

[2] The Royal Society of Canada Report. (1999) A review of the potential health risks of radiofrequency fields from wireless telecommunications devices. The Royal Society of Canada, 225 Metcalfe #308, Ontario, Canada, (http://www.rsc.ca)

[3] Stewart Report (2000). Mobile Phones and Health. Report of Independent Expert Group on Mobile Phones. National Radiation Protection Board NRPB, London, UK (http://www.iegmp.org.uk)

[4] Zmirou Report to the French Health General Directorate. (2001), (http://www.sante.gouv.fr/htm/dossiers/telephon_mobil/index.htm)

[5] Daniells C, Duce I, Thomas D, Sewell P, Tattersall J, dePomerai D. (1998). Transgenic nematodes as biomonitors of microwave-induced stress. Mutat. Res. 399, 55-64

[6] de Pomerai D, Daniells C, David H, Allan J, Duce I, Mutwakil M, Thomas D, Sewell P, Tattersall J, Jones D, Candido P. (2000). Non-thermal heat shock response to microwaves. Nature 405: 417-418

[7] Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, Kiessling M, Hossmann KA. (1997). Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. Neuroscience 81: 627-639

[8] Kwee S, Raskmark P, Velizarov S. (2001) Changes in cellular proteins due to environmental non-ionizing radiation. I. Heat shock proteins. Electro- Magnetobiology 20: 1061-1072

[9] Tibbles LA, Woodgett JR. (1999). The stress-activated protein kinase pathways. Cell. Mol. Life Sci. 55, 1230-1254

[10] French PW, Penny R, Laurence JA, McKenzie DR. (2001). Mobile phones, heat shock proteins and cancer. Differentiation 67: 93-97

[11] Leszczynski D, Joenväärä S, Reivinen J, Kuokka R. (2002), Non-thermal activation of hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: Molecular mechanism for cancer- and blood-brain barrier-related effects. Differentiation, 70, in press

[12] Salford LG, Brun A, Sturesson K, Eberhard JL, Persson BR. (1994). Permeability of the blood-brain barrier induced by 915 MHz electromagnetic radiation, continuous wave and modulated at 8, 16, 50, and 200 Hz. Microsc. Res. Tech. 15: 535-542

[13] Schirmacher A, Winters S, Fisher S, Goeke J, Galla HJ, Kullnick U, Ringelstein EB, Stogbauer F. (2000). Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier in vitro. Bioelectromagnetics 21: 338-345

[14] Fritze K, Sommer C, Schmitz B, Mies G, Hossman KA, Kiessling M, Wiessner C. (1997a) Effect of global system for mobile communication (GSM) microwave exposure on blood-brain barrier permeability in rat. Acta Neuropathol. 94, 465-470

[15] Tsurita G, Nagawa H, Ueno S, Watanabe S, Taki M. (2000) Biological and morphological effects on the brain after exposure of rats to a 1439MHz TDMA field. Bioelectromagnetics 21, 364-371

[16] Neubauer C, Phelan AM, Kues H, Lange DG. (1990). Microwave irradiation of rats at 2.45 GHz activates pinocytotic-like uptake of tracer by capillary endothelial cells of cerebral cortex. Bioelectromagnetics 11: 261-268

[17] Töre F, Dulou PE, Haro E, Veyret B, Aubineau P. (2001). Two-hour exposure to 2-W/kg, 900-MHZ GSM microwaves induces plasma protein extravasation in rat brain and dura matter. Proceedings of the 5th International congress of the EBEA, pp43-45

[18] Pandey P, Farber R, Nakazawa A, Kumar S, Bharti A, Nalin C, Weichselbau R, Kufe D, Kharabanda S. (2000). Hsp27 functions as a negative regulator of cytochrome c-dependent activation of procaspase-3. Oncogene 19: 1975-1981

[19] Concannon CG, Orrenius S, Samali A. (2001). Hsp27 inhibits cytochrome c-mediated caspase activation by sequestering both pro-caspase-3 and cytochrome c. Gene Expression 9, 195-201