Review

Cell phones: modern man's nemesis?

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Abstract

Over the past decade, the use of mobile phones has increased significantly. However, with every technological development comes some element of health concern, and cell phones are no exception. Recently, various studies have highlighted the negative effects of cell phone exposure on human health, and concerns about possible hazards related to cell phone exposure have been growing. This is a comprehensive, up-to-the-minute overview of the effects of cell phone exposure on human health. The types of cell phones and cell phone technologies currently used in the world are discussed in an attempt to improve the understanding of the technical aspects, including the effect of cell phone exposure on the cardiovascular system, sleep and cognitive function, as well as localized and general adverse effects, genotoxicity potential, neurohormonal secretion and tumour induction. The proposed mechanisms by which cell phones adversely affect various aspects of human health, and male fertility in particular, are explained, and the emerging molecular techniques and approaches for elucidating the effects of mobile phone radiation on cellular physiology using high-throughput screening techniques, such as metabolomics and microarrays, are discussed. A novel study is described, which is looking at changes in semen parameters, oxidative stress markers and sperm DNA damage in semen samples exposed *in vitro* to cell phone radiation.

Keywords: *biophysics, cell phone, general health, infertility, radiofrequency electromagnetic waves, RF-EMW*

Introduction

Cell phone usage has increased by leaps and bounds in the past decade and a half. From being a luxury limited to the wealthy, cell phones have become a commodity, virtually indispensable in daily lives. However, every technological advance and its overuse have a negative aspect. The increase in popularity of cell phones is accompanied by a growing concern regarding the harmful effects of cell phone radiation (radiofrequency electromagnetic waves; RF-EMW) exposure on human health. An earlier report of the Independent Expert Group on Mobile Phones, established by the UK government, summarized the relevant studies on the biological effects of RF-EMW (Huber *et al.*, 2000). Since then, a flurry of scientific activities has attempted to define and quantify the adverse effects of RF-EMW. Despite the increasing number of reports concerning the effects of RF-EMW on various biological systems, no satisfactory mechanism has been proposed to explain the effects of this radiation (Feychting, 2005). Although cell phone companies constantly reassure their subscribers about the safety of their product, reports based on animal and human experiments showing adverse effects of cell phones on biological systems have surfaced.

According to various reports, excessive cell phone usage has led to fatigue, headache, decreased concentration and local irritation and burning (Sandstrom *et al.*, 2001). The possible role of cell phone exposure on tumour induction also has been proposed in an epidemiological study (Hardell *et al.*, 2006). Recent studies also have highlighted the role of cell phone exposure on sperm motility, morphology and viability, thus proposing a reduction in male fertilizing potential (Agarwal *et al.*, 2008). Other reports suggest that RF-EMW may lead to DNA damage and chromosomal instability (Diem *et al.*, 2005). Even though the current research may have been inconclusive, it still has been successful in providing preliminary data and identifying trends on both sides of the argument that cell phone exposure may lead to harmful effects on human health. These

studies have been handicapped by many drawbacks in design and methodology. In particular, comparing animal models with humans (Cairnie and Harding, 1981) is impractical. Differences in geometry, size and physiological responses between man and experimental animals imply that the results in animal studies should be interpreted with caution.

Experimental approaches involving animal studies and in-vitro studies, along with high-throughput screening techniques like transcriptomics, proteomics and metabolomics, can augment the validity of epidemiological studies addressing the effect of RF-EMW on reproductive tissues, cells and functions. Recent studies using these approaches have yielded interesting clues on the effect of RF-EMW at the cellular and molecular levels.

This article highlights the adverse affects of RF-EMW on human biological systems by reviewing relevant studies and recent research to aid in deeper understanding of this important health issue. The novel study currently being carried out in the centre is briefly discussed.

An overview of cell phone technology

Telecommunications technology has advanced rapidly and explosively in recent years. The earliest, fully automatic cellular phone systems that were used were called Nordic mobile telephone, now classified as first-generation cellular phones. Introduced in the late 1970s and early 1980s, they were based on analogue technology. The second-generation cell phones that replaced the older analogue type are based on digital technology. These digital models have increased voice capacity, provided faster data transfer speeds, longer battery life, less power use and better signal quality than the first-generation cell phones.

The cell phone technologies that are commonly used nowadays are the global system for mobile communication (GSM) and code division multiple access (CDMA). Both of these technologies are used by cell phone companies in the USA. The GSM technology uses narrow-band time division multiple access (TDMA), whereas CDMA incorporates the wider band that allows more users without interference and better security by providing every user with a unique code.

The third-generation cell phones, which may be available for general use in the near future, consist of universal mobile telecommunications system (UMTS)/wideband code division multiple access (W-CDMA) and the high-speed downlink packet access (HSDPA) phones. The UMTS utilizes a GSM infrastructure with a W-CDMA air interface (the specification of the radio transmission between a mobile phone and the base station), which adds advantages to UMTS over GSM technology. The HSDPA is based on the W-CDMA technology with improved downlink speed that allows even higher data transfer speeds and capacity.

Cell phones in the USA operate on the frequency bands of 850 MHz and 1900 MHz. In most other parts of the world, the frequency bands used are 900 MHz and 1800 MHz. The newer phones offer a quad-band feature, which means that they can operate on the four common frequencies (850/900/1800 and 1900 MHz), and they have the capacity to switch automatically among these four frequencies.

Specific absorption rate (SAR) is the energy flow per unit of mass (watts/kg; W/kg). It is a measurement of the power or heat absorbed by the tissue either in a local area of a human tissue or averaged over the whole body. In the USA, the SAR of cell phones varies from 0.12–1.6 W/kg. Standards are designed to limit the SAR in the body to safety levels. The Federal Communications Commission has set a SAR safety limit of 1.6 W/kg, averaged over a volume of 1 g of tissue, for most parts of the body (see website). Exposure guidelines for RF protection had adopted the value of 4 W/kg averaged over the whole body (SAR_{WB}) 'as the threshold for the induction of adverse thermal effects associated with an increase of the body core temperature of about 1°C in animal experiments' (Barnes and Greenebaum, 2007).

Cell phone radiation output power is measured in units of watts or dBm (decibel referenced to 1 mW). Usually cell phones with higher frequency are assigned less output power. Cell phones commonly used these days operate at an output power of less than 1 W.

Power density is a term for characterizing an RF electromagnetic field. It is defined as the power per unit area and is measured in units of mW/m^2 or $\mu W/cm^2$ (Food and Drug Administration website).

Maximum permissible exposures are based on SAR and power density measurements. The Federal Communications Commission has established safety standards on power density for cell phone base station antenna using 1900 MHz band for the general population an uncontrolled exposure of 1000 $\mu W/cm^2$, and for the 850 MHz band the maximum exposure allowed is about 580 $\mu W/cm^2$, as averaged over any 30-min period. Recent studies demonstrated that RF-EMW emitted from commercially available cell phones have no thermal effects (Straume *et al.*, 2005; Anderson and Rowley, 2007; Yan *et al.*, 2007).

Effect of RF-EMW on general health

This section provides a discussion of the various aspects of human health that have been proposed to be, or actually are, affected by cell phone radiation (RF-EMW) (**Figure 1**).

Effect on cardiovascular system (CVS)

Braune *et al.* (1998) exposed human volunteers to RF-EMW and reported an increase in blood pressure (both systolic and diastolic) on exposure to RF-EMW at 900 MHz for 35 min. Blood pressure increased by 5–10 mmHg, accompanied by a significant decrease in capillary perfusion due to vasoconstriction. They demonstrated, however, that autoregulatory blood pressure mechanisms were intact, as shown by a decrease in heart rate to nullify the increase in blood pressure. In a follow-up study done by the same group to corroborate their previous findings, a statistically significant increase in blood pressure was shown, but the analysis of variance showed that the changes were independent of EMW exposure (Braune *et al.*, 2002). Later, Tahvanainen *et al.* (2004) demonstrated cell phone exposure does not acutely change arterial blood pressure and heart rate.

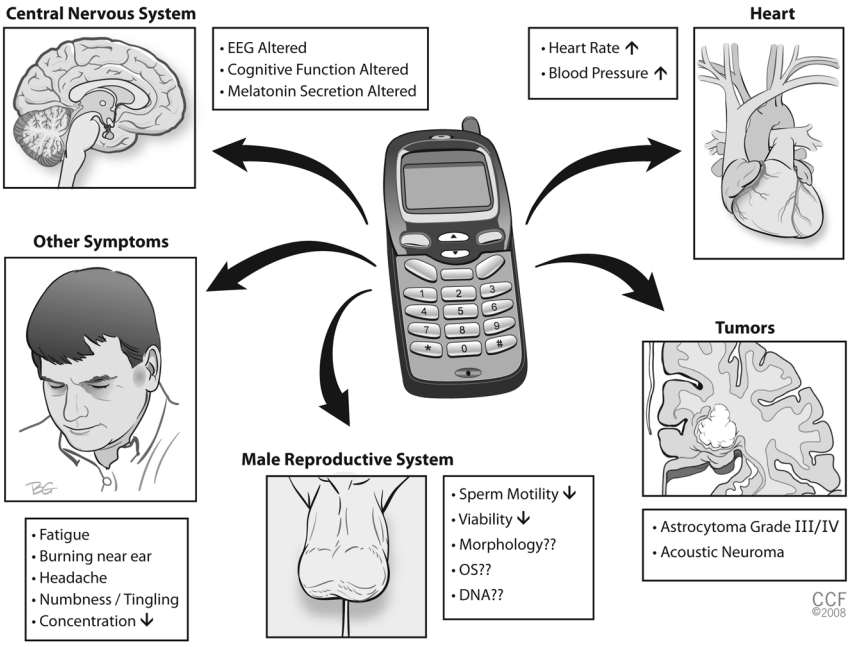

Figure 1. Effect of electromagnetic radiation from cell phone usage on various human systems. OS = oxidative stress.

In an animal study, Ozguner *et al.* (2005) reported increase in oxidative stress in rat myocardium on exposure to 900 MHz RF-EMW (30 min/day, for 10 days).

Effect on sleep

Despite concerns that sleep patterns are disturbed due to excessive cell phone usage, Huber *et al.* (2000) did not report any significant change in sleep quality, sleep latency and rapid-eye-movement sleep latency in healthy young men exposed to 900 MHz for 30 min. The only effect reported was an increase in electroencephalogram power density during the first 30 min of non-rapid-eye-movement sleep, especially α waves and sleep spindles (the type of sleep waves seen with an electroencephalogram). They concluded that the effect of RF-EMW exposure was transitory, limited to the initial phase of sleep and outlasting the RF-EMW exposure. Recently, Perentos *et al.* (2007) found no significant change in resting electroencephalogram on human volunteers exposed to RF-EMW.

Local and general adverse effects

Sandstrom *et al.* (2001), in a questionnaire-based study involving some 17,000 respondents, showed that cell phone usage led to complaints such as warmth on and behind the ear (31%), fatigue (28%), headache (21.4%), decreased concentration (15%), dizziness (10%), memory loss (9%), and tingling and numbness (6.7%). They also concluded that a statistically significant positive trend was shown by warmth and neurasthenic symptoms (headache, fatigue) with calling time and number of calls per day. They proposed that these changes were due to either radiofrequency exposure or thermal effects of EMW. Of all the people who attributed these symptoms to cell phone usage, 45% of them took steps such as reducing calling time, changing cell phone model, using a hands-free kit or using a landline phone to reduce cell phone exposure (Oftedal *et al.*, 2000).

The generation of reactive oxygen species by RF-EMW exposure is still to be proven convincingly, although many groups have provided evidence in animal-based studies. An increase in kidney tissue malonaldehyde and urine *N*-acetyl-β-D-glucosaminidase and decrease in renal superoxide dismutase, catalase and glutathione peroxidase were reported by Oktem *et al.* (2005). Similar results were shown by another investigator (Irmak *et al.*, 2002), who provided evidence in favour of EMW-induced oxidative stress. They showed an increase in superoxide dismutase activity and a decrease in nitric oxide concentrations in sera. Conversely, no change was seen in the concentration of intracellular oxidants [oxidized form of glutathione (GSSG) accumulation, oxidation of thiol] and antioxidants (CuZn-superoxide dismutase, catalase) in cells exposed to radiofrequency radiation (CDMA and GSM, 835–847 MHz for 20–22 h) (Hook *et al.*, 2004).

Cell phones and neurohormonal secretion

Various epidemiological studies have highlighted effects of cell phone usage on neurohormonal secretion. Conflicting results have been reported by different groups regarding the effect of cell phones on melatonin secretion. De Seze *et al.* (1999) reported no change in maximum serum concentration ($P = 0.63$), the time of peak concentration ($P = 0.49$) and area under curve ($P = 0.56$) of the hormonal profile. On the other hand, Burch *et al.* (2002) concluded that subjects with cell phone usage >25 min/day had lower creatinine-adjusted mean nocturnal concentrations of a melatonin metabolite, 6-hydroxymelatonin sulphate (6-OHMS), ($P = 0.05$) and lower overnight 6-OHMS excretion ($P = 0.03$). They concluded that prolonged usage of cell phones may lead to reduced melatonin production. Djeridane *et al.* (2008) demonstrated 900 MHz RF-EMW would not significantly affect endocrine functions in men.

Effects on cognitive function

Preece *et al*. (1999) exposed human volunteers to RF-EMW and reported that the only cognitive function test that altered post-RF-EMW exposure is choice reaction time, leading to an increase in responsiveness. They reported no change in word, number or picture recall or any change in spatial memory. They proposed that the increase in responsiveness was due to a mild local thermal effect of EMW on angular gyrus (the interface between visual and speech centres) or to mechanisms mediated by heat shock proteins. They also concluded that memory is not commonly affected by cell phone exposure as the memory area of the brain (hippocampus) is deep seated in the medial temporal lobe of the brain. Later, Regel *et al*. (2007) demonstrated RF-EMW exposure reduces reaction speed and increased accuracy in working-memory tasks.

Tumorigenesis

Carcinogenic potential of cell phone radiation is one of the most conflicting aspect in various studies conducted by several groups. Following public concern that cell phone exposure may lead to cancer, Hardell *et al*. (2006) conducted an epidemiological questionnaire-based study and concluded that astrocytoma (grade III–IV) and acoustic neuroma did show a positive correlation with cell phone usage, and the odds ratio increased with latency (10 years). However, no increased risk was shown with astrocytoma (grade I–II), non-Hodgkin lymphoma, salivary tumours or testicular tumours. With regard to testicular tumours, they concluded that the risk of seminoma and non-seminoma was not increased, a dose–response effect was not observed, and the location of the cell phone was not associated with testicular cancers (Hardell *et al*., 2007).

Other scientists have concluded that the current evidence for a causal association between cancer and EMW exposure is weak and unconvincing (Colonna, 2005).

Cell phone and effects on male fertility

Pathophysiology

Despite reports from numerous groups suggesting a possible role of cell phone exposure in male infertility, the exact mechanism of the effects of EMW on male reproductive system is yet to be elucidated. Though various effects have been proposed, foolproof experimental evidences are lacking to substantiate it.

Human testes need physiological temperatures 2°C lower than body temperature for optimal spermatogenesis. High-intensity RF has heating properties that lead to thermal effects on the testes. An increase in testicular or body temperature on exposure to EMW may cause reversible disruption of spermatogenesis (Kandeel and Swerdloff, 1988; Jung and Schill, 2000). EMW can also affect reproductive function via an EMW-specific effect (a 'microwave' effect produced by an increase in tissue temperature less than its normal temperature fluctuation) or in combination with the thermal molecular effect (Blackwell, 1979).

As discussed previously, recent studies reported that RF-EMW emitted from commercially available cell phones have no thermal effect (Straume *et al*., 2005; Anderson and Rowley, 2007; Yan *et al*., 2007). However, several views were proposed to elucidate the disruption of metabolic pathways by RF-EMW. Some of these views are based on experimental evidences and some on hypothetical models. Isocitrate dehydrogenase, an important enzyme in the citric acid cycle, is one of the targets of cell phone radiation. Alteration in the enzyme activity leads to decreased production of adenosine triphosphate (ATP) in mammalian cells (Nylund and Leszczynski, 2004). Since sperm motility depends on the active generation of ATP, such a mechanism might cause the decline in sperm motility during RF exposure.

Spermatozoa lose their cytoplasm post-spermiation, leading to the loss of their antioxidant protective mechanism and rendering them inherently vulnerable to induction of DNA damage. They are differentiated to the point that they cannot undergo apoptosis in response to any form of severe genetic damage (Aitken, 1999). In addition, during the process of maturation, spermatozoa are separated from the Sertoli cells, their nursing cells. Several investigators have demonstrated an increase in DNA fragmentation in a variety of human and animal cells following cell phone exposure (Lai and Singh, 1996; Diem *et al*., 2005; Panagopoulos *et al*., 2007). Lai and Singh showed that exposing rats ($n = 16$) for 2 h to pulsed 2-μs pulse width, 500 pulses/s and continuous wave (2450 MHz) leads to an increase in breaks of single-stranded DNA ($P < 0.01$) and double-stranded DNA ($P < 0.01$) in rat brain cells. They proposed that this could be due to either direct EMW-mediated effects or a defect in DNA repair mechanisms.

In contrast, several studies found no effect of EMW on genotoxicity. Stronati *et al*. (2006) demonstrated no effects of RF exposure on DNA strand breakage (assessed by COMET assay), unstable chromosomal alterations (assessed by metaphase analysis) or alterations in the speed of in-vitro cell cycling (assessed by nuclear division index) in lymphocytes in their experiment involving exposure of human blood samples to RF (24 h, 935 MHz). A large-scale in-vitro study conducted by Sakuma *et al*. (2006) concluded that RF-EMW from mobile phone radio base stations do not act as a genotoxicant (at SAR up to 800 mW/kg).

The induction of DNA damage in spermatozoa has been associated with male infertility, early pregnancy loss and morbidity in the offspring, including childhood cancer (Aitken, 1999). Aitken *et al*. (2005) demonstrated that exposure of mice to RF-EMW, 900 MHz, 12 h/day for 7 days led to damage to both the mitochondrial and nuclear genome of epididymal spermatozoa ($P < 0.01$). However, currently no human studies are available demonstrating DNA damage in sperm cells by RF radiation exposure.

Several animal studies have attempted to highlight histological changes in testicular tissue on exposure to RF-EMW. Dasdag *et al*. (1999) demonstrated a decrease in mean seminiferous tubule diameter in rats ($n = 18$) by exposing them to an 890–915 MHz cell phone, 2 h/day for 30 days ($P < 0.05$). However, a similar study carried out later by the same group did not reveal any statistically significant result of cell phone exposure on seminiferous tubular diameter, lipid composition, malonaldehyde

concentration, sperm count or sperm morphology (Dasdag *et al.*, 2003). Ribeiro *et al.* (2007) also did not find any significant adverse effect of cellular phone exposure (GSM 1835–1850 MHz exposure, 1 h/day for 11 weeks) on rat testicular histology and function.

EMW and semen parameters

The effects of cell phone exposure on male fertility have been studied exhaustively in recent years (Deepinder *et al.*, 2007). The effects on sperm concentration, motility and morphology have been evaluated in many animal and human studies, but results are inconclusive. Motility is the only parameter that the majority of studies have shown to be significantly affected. The need to further evaluate the effects of EMW on sperm morphology, viability and concentration still exists.

Dasdag *et al.* (1999) reported a decrease in sperm count; however, the decline was not statistically significant ($P > 0.05$), and they were not able to repeat the same results later in a similar study (Dasdag *et al.*, 2003). Another group reported that exposure of rats ($n = 16$) to a 1.9 Hz cell phone from a distance of 1 cm for 6 h/ day for 18 weeks did not lead to significant decline in sperm concentration. The exposure group had a mean sperm count of $7.45 \times 10^7 \pm 1.03 \times 10^7$ sperm cells/ml, and the non-exposed group had a mean sperm count of $7.7 \times 10^7 \pm 8.11 \times 10^6$ sperm cells/ml ($P > 0.05$) (Yan *et al.*, 2007). In an epidemiological study, researchers concluded that no statistically significant ($P > 0.05$, chi-squared test = 1.48) difference in sperm count resulted from cell phone exposure (Wdowiak *et al.*, 2007). In a study carried out by this centre, a significant decline in sperm count was demonstrated in men who used cell phones for >4 h/ day ($n = 114$, count $50.30 \pm 41.92 \times 10^6$/ml) as compared with those who did not use cell phones at all ($n = 40$, count $85.89 \pm 35.56 \times 10^6$/ml) ($P < 0.0001$) (Agarwal *et al.*, 2008).

As mentioned earlier, motility is the only parameter that consistently has been shown to decline in studies carried out by various groups. In a study involving 371 men presenting for an infertility workup, duration of possession and daily transmission time of cell phones correlated negatively with the proportion of rapid progressive motile spermatozoa ($r = –0.12$ and $r = –0.19$, $P < 0.01$) and positively with the proportion of slow progressive motile spermatozoa ($r = 0.12$ and $r = 0.28$, $P < 0.01$) (Fejes *et al.*, 2005). The same group also concluded that low transmitter (<15 min/day) and high transmitter (>60 min/ day) groups also differed in the proportion of rapid progressive motile spermatozoa (48.7% versus 40.6%, $P < 0.01$). Wdowiak *et al.* (2007) reported that 65.7% of men not using cell phones had >50% (WHO category A + B) sperm motility, whereas only 35.4% of men who frequently used cell phones had >50% (A + B) sperm motility. Agarwal *et al.* (2008) had shown a significant reduction in motility of spermatozoa in men using cell phones >4 h/day versus men not using them at all (67.80 ± 6.16% versus 44.81 ± 16.30%, $P < 0.0001$). In an animal-based study, a significant decrease in sperm motility on exposure to cell phone ($n = 16$, $P < 0.05$) was reported (Yan *et al.*, 2007). The researchers also reported that the majority of sperm cells in the exposure group were dead (live cells 44.88 ± 20.66%); in the control group, the majority of sperm cells were alive with constant, active motility (live cells 70.93 ± 12.94%).

However results of in-vitro studies are conflicting. An in-vitro study divided neat semen samples from healthy volunteers ($n = 27$) into two parts and one part was exposed to 900 MHz EMW for 5 min. Compared with the unexposed sample, the exposed sample was found to have a significant decrease in rapid progressive motility (Grade A, $P = 0.0007$), an increase in slow progressive motility (Grade B, $P = 0.0007$) and an increase in the percentage of immotile spermatozoa (Grade D, $P = 0.0003$) (Erogul *et al.*, 2006). Recently, Falzone *et al.* (2008) studied the effect of pulsed 900 MHz radiation on various kinetic parameters and mitochondrial membrane potential (MMP)of purified human spermatozoa (by percoll density gradient). They found significant decrease in straight-line velocity and beat-cross frequency at an SAR of 5.7 W/kg. However, at an SAR of 2.0 W/kg they found no significant change in any kinetic parameters, including MMP.

Significant changes in sperm morphology were not reported in the animal studies carried out by Dasdag *et al.* (1999, 2003). Similarly no significant ($P > 0.05$) alteration in morphology was reported by another group based on their animal experiment (Yan *et al.*, 2007). However, the same group reported that 80% of the slides in the exposed group showed large clumps of sperm cells that were able only to turn about in their position and were not able to break free. On the other hand, significant data were brought out in a study in which 15.3% of men using cell phones sporadically for 1–2 years had only 10–19% normal spermatozoa, and 15.3% had total azoospermia, whereas men frequently using cell phones for >2 years had only 8.3% normal spermatozoa, and 22.9% showed total azoospermia (Wdowiak *et al.*, 2007).

Transcriptomics and proteomics in elucidation of biological response of cell phone radiation

Research over the last two decades on the effect of RF-EMW has yielded controversial results. It is said that even an extensive epidemiological study might not be sufficient to elucidate the health effects of electromagnetic radiations because of the low sensitivity of this approach. Hence, to validate the results from epidemiological studies, further data from animal and in-vitro studies needs to be analysed. Several lines of evidences suggest that the novel methodologies such as transcriptomics, proteomics and metabolomics could help in the search for clues to the negative impact of cell phone radiation on human health.

High-throughput screening techniques combined with modern bioinformatics could be used to pick up minute variations, like those caused by RF-EMW affecting protein or gene expression, that might be of insufficient magnitude to alter cell physiology or give any phenotypic alteration (**Figure 2**).

Heat shock proteins (Hsp), which are molecular chaperones, comprise a group of highly conserved, abundantly expressed proteins with diverse functions, including the assembly and sequestering of multiprotein complexes, transportation of nascent polypeptide chains across cellular membranes, and regulation of protein folding. Protein phosphorylation is a first line of cellular response to any stimuli by either

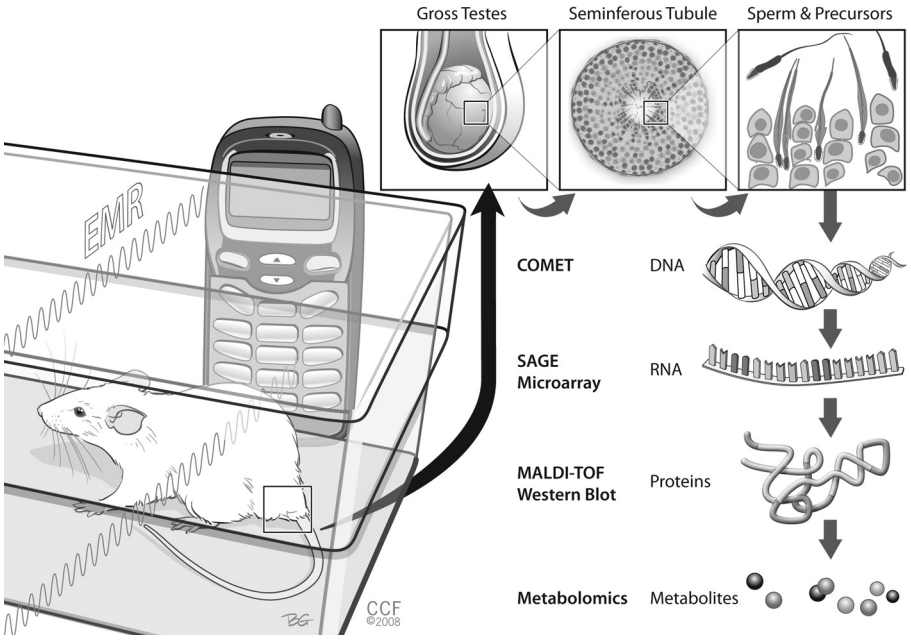

Figure 2. A proposed model to study the effect of cell phone radiation using the high-throughput technologies. These techniques combined with modern bioinformatics could be helpful to find minute variations caused by RF-EMF in protein or gene expression changes that might be of insufficient magnitude to alter cell physiology or give any phenotypic alteration. MALDI-TOF = matrix-assisted laser desorption/ionization time of flight; SAGE = serial analysis of gene expression.

internal or external factors. By using western blots or mass spectrometry, the phosphoproteins could be located after cellular irradiation from a mobile phone to check for any alterations in cell response. By using this approach, Hsp27 was determined to be a molecular target event of RF-EMW (Leszczynski *et al*., 2002).

A study using matrix-assisted laser desorption/ionization-mass spectrometry found statistically significant altered expression levels of 38 various proteins in human endothelial cell lines following GSM 900 MHz irradiation (Nylund and Leszczynski, 2004). Two of the affected proteins were determined to be isoforms of cytoskeletal vimentin and might have an effect on the physiological functions that are regulated by the cytoskeleton.

Results from a study using human lens epithelial cells (HLEC) cell lines indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the augmented Hsp70 protein expression in HLEC occurred without change in the cell proliferation rate (Nylund and Leszczynski, 2004). The induction of Hsp70 by extremely low frequency (ELF) EMW also involves elements of the mitogen-activated phosphokinase (MAPK) family of cell response cascades, which are recognized signal transduction systems present in eukaryotes. MAPK pathways consist of distinct cascades of regulator enzymes that serially activate one another to control the expression of specific sets of genes in response to growth factors, cytokines, tumour promoters and other major biological stimuli. The authors suggest that non-thermal stress response of Hsp70 protein increased on RF exposure might be involved in protecting HLEC from DNA damage and maintaining the cellular capacity for proliferation (Lixia *et al*., 2006).

The phosphorylated Hsp27 (activated) has been shown to inhibit apoptosis by forming a complex with the apoptosome (complex of Apaf 1 protein, procaspase 9, and cytochrome c) or some of its components and preventing proteolytic activation of the procaspase 9 into active form of caspase 9 (Concannon *et al*., 2001). This, in turn, prevents activation of procaspase-3, which is activated by caspase 9. Apaf-1 plays an important role in the induction of apoptosis (Zou *et al*., 1997). Cytochrome c release from mitochondria occurs when there is a DNA-damaging stimuli-induced apoptosis. Together with dATP/ATP, cytochrome c initiates formation of an apoptosome consisting of Apaf 1 oligomers. The Apaf 1 apoptosome recruits and activates caspase 9, which in turn activates the executioner caspases, caspase 3 and caspase 7 (Zou *et al*., 1997). The induction of the increased Hsp27 activation by the RF-EMW exposure might lead to inhibition of the apoptotic pathway that involves apoptosome and caspase 3. It is proposed that such events occurring in RF-EMW-exposed cells that had undergone either spontaneous or external factor-induced transformation or damage could support survival of the transformed/damaged cells (Leszczynski *et al*., 2004).

The exposure of the EA.hy926 human endothelial cell line to 900 MHz RF-EMW induces activation of the p38 MAPK stress response pathway and leads to an increase in expression and phosphorylation of the small stress response protein Hsp27 (Leszczynski *et al*., 2002). Other studies have shown that the phosphorylated form of Hsp 27 has the ability to translocate to the nucleus and to induce changes in gene expression (Geum *et al*., 2002).

The evidence suggests that different types of cells from different species might respond differently to mobile phone radiation

or might have different sensitivity to this weak stimulus. The results from the studies by (Nylund and Leszczynski, 2006) show that gene and protein expression were altered in multiple cell lines in response to 1-h mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were affected differently by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. The magnitude of the genetic background for some stimulus-specific responses was highlighted by some studies comparing different cell lines (Czyz *et al*., 2004). It is postulated that the genetic constitution, as well as carrier frequency of the modulation schemes and exposure duration, may play a substantial role in responsiveness of cells to RF-EMW. These findings might also explain, at least in part, the origin of discrepancies in reproducibility of studies among different laboratories (Nylund and Leszczynski, 2006).

Some evidence has suggested that RF-EMW may change expression of DNA transcription factors and cause changes in cell cycle kinetics. Litovitz *et al*. (1993) have shown that exposure of mouse L929 fibroblasts to 915 MHz at an SAR of 2.5 W/kg induced the expression of ornithine decarboxylase protein, an enzyme important in cell cycle regulation. Natarajan *et al*. (2002) reported that exposure of a monocytic cell line to 8.2 GHz pulse-modulated RF-EMW increased the binding of the nuclear factor kappa light chain gene to its consensus DNA sequence. Later on, relative expression and localization of bone morphogenetic proteins (BMP) and their receptors (BMPR), major endocrine and autocrine morphogens involved in renal development, were investigated by Pyrpasopoulou *et al*. (2004) in newborn kidneys from RF-EMW-exposed pregnant rats. The kidneys of newborns from the RF-exposed rats showed up-regulation of BMP4 and BMPR1A and down-regulation of BMPR2. This study suggests that RF-EMW might interfere with gene expression during early gestation and result in aberrations of BMP expression in the newborn (Pyrpasopoulou *et al*., 2004). RF-EMW has also been reported to affect the expression of *Jun*, a proto-oncogene (Ivaschuk *et al*., 1997). Using serial analysis of gene expression (SAGE), Lee *et al*. (2005) reported that in-vitro exposure of HL-60 cells to pulse-modulated 2.45 GHz RF fields at an SAR of 10 W/kg for 6 h resulted in the differential expression of more than 750 genes. In contrast, many other recent studies have failed to find evidence of RF-field-induced changes in Hsp expression after RF-EMW exposure at frequencies ranging from 900–1950 MHz and SAR from 2–10 W/kg (Capri *et al*., 2004a,b; Laszlo *et al*., 2005). Qutob *et al*. (2006) also reported no evidence relating non-thermal RF field on gene expression using microarray analysis in cultured U87 MG cells.

Studies done on *Drosophila melanogaster* developmental potential by exposure to non-thermal radiation from the GSM mobile phone found increased numbers of offspring and elevated Hsp70 levels (Weisbrot *et al*., 2003). This study also reported increased serum response element DNA-binding and induction of the phosphorylation of the nuclear transcription factor ELK-1 by cell phone radiation. The rapid induction of Hsp70 within minutes by a non-thermal stress, together with identified components of signal transduction pathways, could provide sensitive and reliable biomarkers that could serve as the basis for practical mobile phone safety guidelines (Weisbrot *et al*., 2003).

The indications to date that certain genes are influenced by EMW suggests that genome-wide scans of the transcriptome are necessary. Among the several technologies used for genome-wide gene expression analysis, SAGE is one promising method that seems particularly applicable for EMW research. SAGE has been used in many biological and medical studies involving various eukaryotic species. So far, more than 19 million copies of SAGE tags have been collected from humans (Wang, 2006).

In a recent study by Remondini *et al*. (2006), which was part of the Fifth Framework Programme project REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive In-Vitro Methods), six human cell types, immortalized cell lines and primary cells were exposed to 900 and 1800 MHz. RNA was isolated from exposed and sham-exposed cells and labelled for transcriptome analysis on whole-genome cDNA arrays. NB69 neuroblastoma cells, T lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. In EA.hy926 endothelial cells, U937 lymphoblastoma cells and HL-60 leukaemia cells, between 12 and 34 genes were up- or down-regulated (including bcl-2-associated transcription factor *BTF* gene). The findings conclude that analysis of the affected gene families does not point towards a stress response, and no consistent RF-EMF signatures could be detected. However, following RF-EMW exposure, some but not all human cells might react with an increase in expression of genes encoding ribosomal proteins and therefore up-regulating the cellular metabolism (Remondini *et al*., 2006).

Theoretical approaches also have been proposed to elucidate the mechanism behind the stimulation of biosynthesis by EMW (Blank and Goodman, 2008). Electrons have been shown to move in DNA and biochemical reactions could be modulated by EMW (Blank, 2005). Interaction with electrons could explain the activation of DNA by weak, low-frequency EMW, as well as the more energetic high frequencies. Evidence from biochemical reactions suggests that electromagnetic fields can accelerate electron transfer. Interaction with electrons could displace electrons in H bonds that hold DNA together, leading to chain separation and initiating transcription. The electron transfer would favour separation of base pairs, and DNA geometry is optimized for disaggregation under such conditions. The initial interaction could involve the displacement of electrons in the H bonds that hold DNA together, thereby causing chain separation and initiating transcription and translation. EMW-initiated DNA separation can set in motion the interconnected biochemical signalling pathways that are activated in the stress response (Blank and Goodman, 2008). The effects of low-frequency EMW on Na/K-ATPase activity (Blank, 2005) to generate ATP is another pertinent field to explore in the context of spermatozoal motility. The Na/K-ATPase is an enzyme of the plasma membrane of most animal cells that uses the free energy from the hydrolysis of ATP to mediate the exchange of cytoplasmic Na^+ for extracellular K^+ in a 3:2 ratio (Kaplan, 2002; Sanchez *et al*., 2006). The Na/K-ATPase plays a key role in numerous cell processes that depend directly or indirectly on the transmembrane gradients of Na^+ and K^+. The enzyme is essential in maintaining cell osmotic balance, volume, pH and the cell resting membrane potential and in providing the chemical energy for the secondary Na^+-coupled transport of other ions, solutes and water across the cell membrane (Skou and Esmann, 1992). This enzyme has an important role, along

with Na^+/H^+ exchanger, in human sperm motility (Woo *et al*., 2002; Sanchez *et al*., 2006) These cellular pathways should be further analysed in the context of EMW. More recently Friedman *et al*. (2007) found significant increase in plasma membrane NADH oxidase activity of mammalian cells (HeLa cells) after exposure to 875 MHz EMF.

Although the use of the discovery science approach employing high-throughput screening techniques will not yield foolproof evidence of a health hazard or its absence, it will be essential in unravelling the complexities of the biological effects potentially exerted by RF-EMF exposure.

Cleveland Clinic pilot study

To validate the results of recent epidemiological studies and to establish a cause and effect relationship between cell phone usage and decrease in semen parameters, a novel in-vitro experiment was designed. Semen samples were exposed to EMW from a commercially available cellular phone (GSM network, 850 MHz,), and the effect of EMW on semen parameters, DNA integrity [using TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling assay] (Tesarik *et al*., 2006; Ozmen *et al*., 2007) and disturbance in reactive oxygen species metabolism was assessed post exposure. In this study, healthy donors were enrolled to provide semen samples. The semen sample obtained from each volunteer was divided into two parts: EMW-exposed group and control group. Environmental condition was monitored throughout the experiment. The frequency emitted by the cell phone was also confirmed with help of a radiofrequency spectrum analyser. One portion of the sample was exposed to radiation from a commercially available cell phone. A second portion was kept non-exposed for the same time duration. Measurement of sperm concentration, motility and viability was carried out as described by the World Health Organization (1999). Samples also were assessed for reactive oxygen species, total antioxidant capacity and DNA damage (Agarwal *et al*., 2008).

Conclusion

As highlighted above, many aspects of human health have been proposed to be affected by cell phone exposure. Ranging from mild local warmth to possible tumour induction, EMW have been suspected of involvement in many health concerns. At this time, evidence is lacking to strongly prove or disprove any of the proposed harmful effects of EMW. However, the significance of these studies and their possible implications in the future cannot be ignored. Findings and trends available from these studies provide a strong indication to carry out further studies to establish a clearer and more evidence-based conclusion.

Both human and animal-based studies have provided a hint that EMW may be involved in the pathogenesis of male infertility, but considerable work is required to provide scientific support for this view. More importantly, studies must be carried out in human semen samples as data from animal studies are limited in their applicability in humans.

High-throughput screening techniques may be an important tool to evaluate the molecular effects of EMW on the biological system. Not only will these techniques provide evidence in support of previous studies, they also will open opportunities for groundbreaking research in this area.

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