Possible Mechanisms to Explain Biological Effects from Low Level RF Exposure (i.e., Wireless Communication Signals)

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Abstract

With the popularity of mobile phones and other wireless communication devices has come a level of concern regarding adverse health effects due to long-term low-level radiofrequency (RF) exposure. In addition to experimental studies, a significant number of papers have proposed theoretical mechanisms to explain how RF energy might interact with biological tissue. Many of these describe “non-thermal” pathways, although with respect to wireless communication frequencies and signal types the most plausible mechanism of interaction based upon current evidence and scientific opinion is based upon thermal interactions. In addition, several reports in the literature also demonstrate that cells in culture can be responsive to low levels of RF exposure. The present study reviews potential mechanisms and presents data to support thermal interactions as the primary mechanism for the biological effects of RF exposure for the frequencies and signal types used for wireless communication.

Introduction

A level of public concern has been raised over possible adverse health consequences of long-term low-level radiofrequency (RF) exposure from mobile phones and other wireless communication equipment. In response, the World Health Organization (WHO) has developed a research agenda [1] and collected a literature database [2] to help guide and coordinate global funding and research to support a successful risk assessment. The current literature database contains several hundred reviews and over 2300 peer reviewed published papers, many of which propose nonlinear mechanisms describing how RF energy might interact with biological tissue at “non-thermal” levels. The current weight of evidence and scientific consensus, however, generally support a limited number of basic mechanisms for the interaction of RF with biological tissue [3].

Several studies in the literature have also reported that “non-thermal” levels of RF exposure to cells in culture can result in effects on proliferation [4-7] as well as related endpoints including expression of immediate early genes and MAP kinases [8, 9], heat shock proteins [10], and other targets [11-16]. One simple assay to evaluate whether these responses may be due to small temperature increases in the culture medium is to look at cell cycle progression. Transit through the cell cycle is a tightly controlled process with a sequential progression from G₁ to S to G₂ to M. Proliferation can halt at the G₁/G₀ restriction point when cells reach confluence or when the environment is not optimal for continued growth. Proliferation can also halt at either G₁ or G₂ checkpoints in response to environmental stress or insult until damage is repaired.

Since animals (including humans) have elaborate thermoregulatory systems and will commonly experience temperature excursions during daily activity (e.g., exercise, fever, sleeping, circadian rhythm, eating), small thermal insults are not likely to cause adverse effects. However in experimental systems, prior evidence suggests cell lines [19-24] and yeast [25] may respond to slightly elevated culture temperatures, far lower than the more dramatic temperature increases required to trigger stress responses [26, 27]. Characterization of the effects of small temperature elevations in experimental models may not only add insight into existing reports in the RF literature, but also have significant relevance to other areas of radiation biology including heat radio-sensitization of tumors and UV damage (temperature effects on skin aging).
Results

Growth curves of cells cultured at increasing temperatures are shown in Figure 1. C3H 10T ½ cells showed accelerated growth at 38°C as well as increased sensitivity to 39 and 40°C than human glioma cell lines U87MG and LN71. All cell lines showed significant growth delays at 40°C, with LN71 cells responding almost immediately after temperature shift while in U87MG cells decreased only after 24 hours.

The short term rate of transit through each phase of the cell cycle was examined every 2 hours for the 12 hours following initial temperature shift (on day 3 of culture) using dual-parameter flow cytometry. Figure 2 (fraction of cells in the BrdUrd-negative G₁ compartment) shows C3H 10T ½ cells shifted to 38°C are transitioning from G₁ into S phase more rapidly than 37°C controls. In contrast, transit kinetics of both human glioma cell lines at 38°C was similar to 37°C controls. C3H 10T ½ cells also showed more sensitivity to increased temperature (40°C) than U87MG and LN71 cells. Figure 3 (fraction of cells in the BrdUrd-negative G₂ compartment) shows C3H 10T ½ fibroblasts are transitioning through S phase into G₂ more rapidly than 37°C controls. In contrast, transit kinetics of both human glioma cell lines at 38°C was similar to 37°C controls. C3H 10T ½ cells again showed more sensitivity to increased temperature (39 and 40°C) than U87MG and LN71 cells. Relative DNA (Figure 4) confirms accelerated transit through S phase by C3H 10T ½ fibroblasts shifted to 38°C as compared with 37°C controls, with no such acceleration observed in the human glioma cell lines. C3H 10T ½ fibroblasts again showed increased sensitivity at higher temperatures. A similar pattern was observed with G₂ and M phases (data not shown). Figure 3 (fraction of cells in the BrdUrd-negative G₂ compartment) shows C3H 10T ½ cells shifted to 38°C did not show accumulation in G₂, presumably because cell cycle arrest due to reaching confluence occurs in G₁/G₀. When shifted to 39°C, In C3H 10T ½ cells again showed no checkpoint halt, reflecting a steady but slower transit through the cell cycle. However when cultured at 40°C, C3H 10T ½ cells showed clear evidence of G₂ checkpoint halts, supporting a mechanism that delay is due to temperature stress.

The expression of c-fos is known to respond to a variety of stress agents, and was evaluated in C3H 10T ½ cells to help confirm whether the effects observed on cell cycle transit due to temperature shifts were due to stress. Figure 5 shows that in control (37°C) cells there is normally a 1.5-2 fold increase in c-fos expression upon transitioning out of the exponential growth phase and reaching confluence. In cells cultured at 38°C (data not shown) this pattern was similar, although since confluence was reached earlier the increased expression of c-fos also occurred earlier in parallel with confluence. In C3H 10T ½ cells cultured at 39 and 40°C, however, a more pronounced elevation of c-fos was observed prior to the cells reaching confluence.

Discussion

The results indicate that cells in culture can be differentially responsive to small changes in temperature. In particular, murine C3H 10T ½ fibroblasts were more sensitive to small temperature changes than human glioma cell lines U87MG and LN71. All cell lines showed unique responses and sensitivities to higher (39-40°C) temperature. Corroboration of these effects is supported by the use of flow cytometry to follow cell transit through the different cell cycle compartments. Whether these responses represent a genuine biological response or an artifact of the cell culture system (e.g., media stirring, gas saturation) is not known. Assuming they are true biological responses, it cannot be assumed they would translate into an adverse health consequence in humans as temperature excursions of this magnitude are common during daily activity. However, many human systems have been shown to respond to small temperature increases. The phenomenon of microwave hearing in response to high peak power, low average microwave energy has been well documented since the 1970’s [28]. The actual temperature elevation associated with thermoelastic expansion of the brain is on the order of 0.000001°C [29]. Action potentials measured from isolated neurons have also been shown to be responsive to temperature changes on the order of 0.2°C [30]. More recent studies have reported very small skin warming (+/- 0.6°C) can have effects on response speed in psychomotor vigilance tests [31] and certain temperature sensitive populations of neurons of the preoptic and anterior hypothalamic can respond to temperatures via sensitive cation channels [32].
Figure 1. Growth curves of C3H 10T ½ cells (top panel), U87MG cells (middle panel) and LN71 cells (bottom panel) cultured at 37, 38, 39, or 40°C. Data point = mean +/- SD from three independent experiments.

Figure 2. Fraction of C3H 10T ½ cells (top panel), U87MG cells (middle panel) and LN71 cells (bottom panel) in BrdU-negative G1 compartment. At 72 h at 37°C, flasks were shifted to designated temperatures and assayed every 2 hours for 12 hours. Data point = mean +/- SD from three independent experiments.

Figure 3. Fraction of C3H 10T ½ cells (top panel), U87MG cells (middle panel) and LN71 cells (bottom panel) in BrdU-negative G2 compartment. At 72 h at 37°C, flasks were shifted to designated temperatures and assayed every 2 hours for 12 hours. Data point = mean +/- SD from three independent experiments.

Figure 4. Relative DNA content (ratio of PI stained BrdU-negative S to BrdU-negative G1 fraction) in C3H 10T ½ cells (top panel), U87MG cells (middle panel) and LN71 cells (bottom panel) shifted to the test temperature of 37, 38, 39, or 40°C. Data point = mean +/- SD from three independent experiments.
Figure 5. Northern blot analysis of c-fos expression during 96 hour culture of C3H 10T½ fibroblasts at 37, 39, and 40°C

References