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Effects of 3G cell phone exposure on the structure and function of the human cytochrome P450 reductase

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ABSTRACT

Cell phones increase exposure to radiofrequency (RF) electromagnetic fields (EMFs). Whether EMFs exert specific effects on biological systems remains debatable. This study investigated the effect of cell phone exposure on the structure and function of human NADPH-cytochrome P450 reductase (CPR). CPR plays a key role in the electron transfer to cytochrome P450, which takes part in a wide range of oxidative metabolic reactions in various organisms from microbes to humans. Human CPR was exposed for 60 min to 1966-MHz RF inside a transverse electromagnetic cell (TEM-cell) placed in an incubator. The specific absorption rate (SAR) was 5 $W \cdot kg^{-1}$. Conformation changes have been detected through fluorescent spectroscopy of flavin and tryptophan residues, and investigated through circular dichroism, dynamic light scattering and microelectrophoresis. These showed that CPR was narrowed. By using cytochrome C reductase activity to assess the electron flux through the CPR, the Michaelis Menten constant (Km) and the maximum initial velocity (Vmax) decreased by 22% as compared with controls. This change was due to small changes in the tertiary and secondary structures of the protein at 37 °C. The relevance of these findings to an actual RF exposure scenario demands further biochemical and in-vivo confirmation.
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1. Introduction

Cellular telecommunication is inseparable from our everyday life. Numerous studies have investigated the potential impact of Global System for Mobile Communication (GSM) on biological systems with several positive and negative outcomes. The biological effects of RF exposure may interact at different levels of biological systems such as at the organic, tissue, cellular and macromolecular level. It is reasonable to assume that any possible biological damage could start at a molecular level involving biological macromolecules. It has been suggested that the main target of RF could be cell membranes and proteins (channel proteins, signal transducers, etc.) or enzymatic complexes located within membranes [1]. However, few investigations have reported as to the possible subcellular effects of RF exposure emitted by 3G cell phones. Proteins are macromolecules found in a living cell and play a crucial role in almost every biological process.

One first step and simplified way is to study the direct effect of RF exposure on protein conformation and function. Some studies suggest that non-thermal microwave exposures could alter protein expression and/or induce conformational changes [2,3]. In these macromolecules, charge distribution may be the cause or the consequence of a conformation change and functional modification of the biological activity could result therefrom. [4–6]. Among such enzymatic complexes, embedded in biological membranes and potentially affected by EMF are cytochrome P450 dependent monooxygenases as well as enzymatic proteins (NADH cytochrome b5, b5 reductase). The above complexes are the key enzymes of phase I reactions, which initiate the metabolism of lipid soluble xenobiotics [7]. Some physiological effects attributable to RF radiation may eventually be traced to alterations in cell membrane function. As it is involved in electron transportation to cytochrome P450, the human NADPH-CPR is suitable as a model for intensive investigation.

Abbreviations: RF, radiofrequency; CPR, cytochrome P450 reductase; EMFs, electromagnetic fields; DLS, dynamic light scattering; 3G, 3rd generation of mobile telephony; TEM-cell, transverse electromagnetic cell; SAR, specific absorption rate; GSM, global system for mobile telephony; WCDMA, wideband code division multiple access; UMTS, Universal Mobile Telecommunication System; FDTD, Finite Difference-Time Domain; Trp, tryptophan.

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In order to better understand the biological effects of RF, different techniques like fluorescence, circular dichroism (CD), dynamic light scattering (DLS) and microelectrophoresis can be used to investigate possible RF-induced modifications. Many biological molecules absorb photons, but few fluoresce, particularly among aromatic amino acids. Two of the most important types of biomolecules that do fluoresce (i.e., those which have proved useful in analytical procedures) are tryptophan and flavins (FAD and FMN) [8]. Both of these intrinsic fluorescence markers are present in the NADPH-cytochrome P450 reductase, allowing the examination of structural changes which take place in this protein after exposure. For these reasons, we chose to study the effects of RF EMF on human CPR. Human CPR is a complex multi-domain membrane-bound diflavo-protein and a key electron donor to P450-mediated microsomal electron transport system. As underlined by Wang et al. [9], microsomal electron transport mediated by cytochrome P450 is responsible for oxidative metabolism of both endogenous and exogenous compounds. Electron transport is mediated by a multicomponent monooxygenase system, in which reducing equivalents of NADPH are in fine transferred to molecular oxygen [10,11]. A simple form of the monooxygenase system consists of CPR and one of many cytochrome P450 isozymes [12,13]. Both CPR and microsomal cytochromes P450 are integral membrane proteins; together with CPR, the only other mammalian enzyme containing both FMN and FAD as prosthetic groups is nitric-oxide synthase and various isoforms thereof. Other physiological electron acceptors of CPR include microsomal heme oxygenase [14], cytochrome *b*5 [15] and, although not used in normal physiological reactions, CPR is capable of transferring reducing equivalents to cytochrome C [16]. As only two protein components are required to catalyze the hydroxylation of a number of substrates, this system represents a simple model for other more complex electron transport systems.

Fluorescence spectroscopy reveals microstructural details of a chromophore's environment at very low chromophore concentrations. The fluorescence properties of proteins are highly individual, a sort of fingerprint of the protein's structure, and their behavior is largely dependent on folding or unfolding. The CPR molecule is composed of four structural domains: (from the *N*- to *C*-termini) the FMN-binding domain, the connecting domain, and the FAD- and NADPH-binding domains. CPR contains two flavin domains (FAD and FMN) and 9 tryptophan residues as internal fluorescence probes. Electrical charges are differently distributed in all macromolecules. For many biomolecules such as proteins, electrostatic interactions influence their conformations and functions. Several studies demonstrate that hydrodynamic radius and zeta potential can provide information about the change in conformation and surface modification of the biomolecules and particles in solution [17–19]. Since the protein retains its folded conformation, hydrodynamic size and zeta potential (charge) will vary with protein conformation. These parameters can be measured by DLS.

The purpose of the present investigation was, therefore, to determine if exposure to RF signals used by 3G cell phones could alter the structure and activity of CPR.

2. Materials and methods

2.1. Reagents and chemicals

Highly purified detergent solubilized human CPR was purchased from Sigma-Aldrich (St. Louis, MO, USA). CPR was expressed in baculovirus infected cells containing human cDNA. Its molecular mass was ~78.2 kDa, (95% purity by affinity chromatography). CPR was diluted in 100 mM potassium phosphate buffer, filtered to clear off large sized aggregates and subjected to protein content determination by Modified Lowry assay. 3-ml samplings were poured into a (12.5 × 12.5 × 45 mm) polymethylmetacrylate (PMMA) cuvette (VWR, Fontenay-sous-Bois, France) for RF exposure and spectrometric analyses. Horse heart cytochrome C was also purchased from Sigma. Purified water with a typical resistivity of 18 MΩ·cm was produced from a Milli-Q purification system (Millipore, Les Ulis, France). All other reagents were of analytical quality, and all aqueous solutions were prepared with Milli-Q water with a Millipore water purification system.

2.2. RF exposure system

Exposures were carried out in a small TEM-cell placed in a temperature-controlled incubator (Scheme 1). The TEM-cell, designed and built by the XLIM Research Institute (CNRS-University of Limoges, France), is an open RF chamber where the intensity of the exposure inside the chamber depends on the input and reflected RF powers of the system [20–22]. To minimize the reflection of the RF power, the TEM cell was loaded with a 50-Ω load (HTF-525-NM, Trilithic Inc. USA). 3G cell phones use wideband code division multiple access (WCDMA) standard RF signals, also called Universal Mobile Telecommunication System (UMTS), which operate in the frequency range of 1920 to 2170 MHz. The WCDMA modulation is a non-periodic cocktail of signals with 5-MHz bandwidth. The 1966 MHz RF signal modulated by a specific WCDMA signal "cocktail" was generated by a Generic UMTS Signal generator (GUS-6960, Universität Wuppertal, Germany). As the output power level of the generator is limited, a RF power amplifier (OPHIR, 5303069, USA) was connected to guarantee the necessary RF power. A built-in step attenuator of the UMTS generator controlled the exposure level of the sample. The absorbed RF power or SAR in the sample depends on the RF exposure intensity. A polymethylmetacrylate cuvette (4.5 ml, Brand ®, Wertheim, Germany) containing 3 ml of 130 nM CPR was placed in the center position of the TEM-cell and exposed to a 1966 MHz RF signal (Fig. 1 (A)). The temperature of the incubator (Certomat HK, France) was controlled within ±0.5 °C during the study. The temperature of the samples inside the cuvette under RF exposure was measured by non-perturbing optical temperature probes (Luxtron model 790, Lumasense, USA). The non-perturbing feature of thermal probes is very important in RF studies. Any metallic wire can enhance the local SAR in the sample. Therefore, during RF exposure, metallic wires must be avoided.

Experimental RF dosimetry - The SAR of the solution was determined experimentally by temperature measurements [20,23]. The classic method of experimental SAR measurement is based on RF energy absorption in the water solution to produce a rise in temperature. The SAR is linear with the initial slope of the temperature increase. Therefore, the SAR was calculated from the temperature rise produced by short RF exposure according to the equation:

$$\mathrm{SAR}\left(\mathrm{W}\cdot\mathrm{kg}^{-1}\right) = C\cdot(dT/dt) \quad (1)$$

where dT is the temperature rise in K, dt is the duration of RF exposure in seconds and C is the heat capacity of the solution ($C = 4187\ \mathrm{J}\cdot\mathrm{kg}^{-1}\cdot\mathrm{K}^{-1}$). The SAR measurement was performed at the highest RF input power level available in the system to achieve a great enough temperature increase during a short RF exposure duration. For SAR measurements, the RF input power of the TEM-cell was 3.88 W. The incident and reflected RF powers were monitored by a power meter system with a built-in directional coupler (Rohde-Schwarz FSH-Z44, Germany). The temperature rise in the solution was measured by fiber-optic non-perturbing temperature probes. The non-perturbing fiber-optic probe was inserted into 3 ml of solution at the cuvette cross section center at different heights from the bottom plate. The SAR was calculated by the temperature rise following 30 s of RF exposure from several measurements.

The SAR in the solution was measured at 4, 8, 12, 16, 20 and 24 mm from the bottom of the cuvette. The average SAR over the whole sample normalized to 1 W input RF power was $50 \pm 13\ \mathrm{W}\cdot\mathrm{kg}^{-1}$ (Table 1).

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