Effects of cell phone radiofrequency exposure on the human cytochrome P450 reductase

Shazia Tanvir, Gyorgy Thuroczy, Brahim Selmaoui, Viviane Silva Pires-Antonietti, Pascal Sonnet, Philippe Lévêque, René De Seze, Sylviane Pulvin

Titre (***français***): Effets de l'exposition aux radiofréquences de téléphone portable sur le cytochrome P450 réductase humain

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Shazia Tanvir [a]*, Gyorgy Thuroczy [b], Brahim Selmaoui [c]**, Viviane Silva Pires-Antonietti [d]***, Pascal Sonnet [e]***, Philippe Lévêque [f] ****, Rene de Seze [g]**, Sylviane Pulvin [*h]**

[*]*Laboratoire de Génie Enzymatique et Cellulaire,UMR 6022 CNRS, Université de Technologie de Compiègne, BP 20529-60205 Compiègne Cedex. France, {a,h}tanvirsh@utc.fr, sylviane.pulvin@utc.fr*

[**]*Unité de* toxicologie *expérimentale, Institut National de l'Environnement Industriel et des risques, Verneuil-en-Halatte, BP2 60 550 (INERIS), France, {b,c,g} gyorgy.thuroczy@ineris.fr,brahim.selmaoui@ineris.fr, rene.de-seze@ineris.fr*

[***]*Université de Picardie Jules Verne, Laboratoire des Glucides, CNRS FRE 3517, équipe THERA, Faculté de Pharmacie, 1 rue des Louvels, 80037, Amiens, France {d,e}vs-phar@u-picardie.fr, pascal.sonnet@sa.u-picardie.fr*

[****]*Université de* Limoges - XLIM - *UMR 6172 CNRS 123, av. Albert Thomas F-87060 Limoges cedex, France{f}philippe.leveque@unilim.fr*

Mots-clés (en français) : Radiofréquence (RF), téléphone portable, UMTS, cytochrome P450 réductase

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Résumé

Accelerated and widespread of cell phones has increased the human exposure to radiofrequency electromagnetic fields (EMFs) and raises serious concerns about the biological and health-related effects of RF radiation. The present study aimed at the effect of cell phone RF (1.9 GHz) emitted by Universal Mobile Telecommunication System (UMTS), the third generation (3G) of cell phones on structure and function of human cytochrome P450 reductase. The ability of the cytochrome P450 reducatese to transfer the electron was measured by an exogenous electron acceptor cytochrome C with UV–vis spectrophotometer. Structural changes were studied by fluorescence spectroscopy, dynamic light scattering, Circular dichroism and capillary electrophoresis (Zeta potential). The results indicated that UMTS-like one hour RF exposure at 1 W/kg SAR slightly altered electron transfer ability and structure of cytochrome P450 reductases.

Introduction

The accelerated cell phone usage has increased human exposure to radiofrequency (RF) electromagnetic fields (EMFs). The question whether these EMFs exert any specific effect on biological systems is still debatable. In the present study the effect of 1.9 GHz RF emitted by Universal Mobile Telecommunication System (UMTS, 3G) on structure and function of human cytochrome P450 reductase (CPR) in-vitro was investigated. CPR is an electron transport enzyme of the microsomal redox chain of the endoplasmic reticulum. Our research strategy was to study the effects of RF exposure at the molecular level using a solution of highly purified human reductase. It is reasonable to assume that any possible biological damage could start at molecular level involving biological macromolecules. Proteins are the macromolecules found in a living cell and play a crucial role in almost every biological process. One simple approach to this problem is studying the effect of irradiation on protein function and conformation. We have chosen human CPR as a protein model because it is a key electron donor to P450-mediated microsomal electron transport system. This enzyme appears to be universally present [1, 2]. CPR is a complex multidomain protein consists of diflavin and tryptophan residues making this enzyme suitable to examine structural changes, possibly taking place after exposure, by fluorescent spectroscopy.

The purpose of the present investigations was to determine if the short term in vitro exposure to UMTS signals as used by 3G cell phones could alter structure and activities of CPR.

Materials and Methods

The Recombinant human CPR (Sigma, St. Louis, MO) was exposed to RF in two TEM-cell systems (control and exposed) set in a temperature controlled incubator. The CPR were diluted in 100 mM potassium phosphate

buffer and filtered to clear off large sized aggregates and subjected to protein content determination by Modified Lowry assay. Horse heart cytochrome C was also purchased from Sigma. RF signal with WCDMA modulation was generated by Generic UMTS signal generator (GUS 6960S, University of Wuppertal connected to a high power amplifier (Ophir, 5303069, USA) to guarantee the power requirements for the study [3]. The 3 mL CPR solution contained in a polymethylmetacrylate cuvette was inserted into the TEM-cell for one hour. The specific absorption rates (SAR) was 1 W/kg [4]. The SAR in the solution was determined by elevation of temperature measurement by non-perturbing optical temperature probes (Luxtron 790F 590F, USA) following short time RF exposure.The change of temperature inside the cuvette during RF radiation of 1 W/kg SAR was below 1°C. The treated sample was matched with unexposed control which was kept in the same experimental conditions. The ability of the CPR to transfer the electron was measured by a non-physiological electron acceptor cytochrome C with UV–vis spectrophotometer. Structural changes were studied by fluorescence spectroscopy, circular dichroism, capillary electrophoresis and dynamic light scattering.

Results and discussion

The results indicated that electron transfer ability and structure of cytochrome P450 reductase was altered under WCDMA modulated RF exposure (Table 1). After 1 hour of irradiation, CPR structural changes are irreversible and consistent with a new thermodynamically stable structure of the enzyme, providing reproducible spectroscopic and kinetic data. In this context, comparative CPR activities measured via the reduction of cytochrome c, the surrogate electron acceptor most commonly used for measuring diflavin reductase activity. The RF exposure produced 22% decrease in both Km and V_{max} as compared to control values at 1W/kg after one hour of exposure. These results suggested that EMFs has reduced enzyme activity means lowered the rate of electron transport under the given set of experimental conditions. Among such enzymatic complexes, embedded in the 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[5]. Since many of the physiological effects attributable to RF radiation may be eventually traced to alterations in cell membrane function. The membrane protein like human CPR is suitable as a model for intensive investigation as being involved in electron transportation to cytochrome P450.

Conclusions

Learning how UMTS exposure affects the structure and function of the CPR is essential for understanding the RF mediated bio-effects on the electron transport chain and eventually on metabolism. The relevance of these

findings to real RF exposure scenario however demands further biochemical and in-vivo confirmation. If CPR of a healthy human exposed to these frequencies transfer less electron to their redox partners, the metabolic abilities especially xenobiotic transformation could be disturbed. It is quite possible that the effects of RF radiation on biological systems may be either masked by repair and regulation mechanisms or transient.

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Table.1. Summarized results

Experiments	**Results**
Tryptophan emission fluorescence	Exposure of CPR to RF radiation resulted in the loss of intrinsic tryptophan fluorescence. The absence of sift clearly argues that the environment of most of the molecules is not altered significantly by the exposure to RF.
Flavin emission fluorescence	The exposed protein showed enhancement of flavin fluorescence intensities. These changes in the fluorescence properties are likely to reflect loosing of protein-flavin contacts.
Circular dichroism	The spectral difference and data indicate that structure of exposed protein has slightly more secondary structure. The data showed that RF exposure induced small but visible changes in the secondary structure of protein.
Dynamic Light Scattering	The average hydrodynamic diameters have shown minor changes in the unexposed (15±2) to exposed (10±3) samples.
Zeta potential	A shift in the Zeta potential is observed at pH 7, from -21 ± 4 mV to -10 ± 3 mV, indicating that the total surface charge at the sliding plane becomes, as a mean value, more positive in the exposed sample. Perhaps, the topological distribution of charges and their interaction with the electromagnetic fields can cause conformational and functional modification of biological activity.
Cytochrome C reductase activity	The RF exposure produced 22% decrease in both Km and V_{max} as compared to control values. The inhibition pattern looks like uncompetitive inhibition, enzyme's apparent affinity for the substrate is increased (K_m is lowered) and decreases the maximum enzyme activity (V_{max}), as it takes longer for the product to leave the active site.