Effects of Microwaves from GSM Mobile Phones on the Blood-brain Barrier and Neurons in Rat Brain

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Abstract

Our group has since 1988 studied the effects of different intensities and modulations of 915MHz RF in a rat model where the exposure takes place in a TEM-cell during various time periods and post exposure recovery times. The power fed into TEM-cells was 0.125, 1.25, 12.5 or 125mW corresponding to whole body SAR (determined experimentally): 0.2, 2, 20 or 200mW/kg.

The rats were awake and not restrained during exposure and after the recovery period the animals were anaesthetized and sacrificed by perfusion-fixation with 4% formaldehyde. Paraffin embedded 5 μm. thick brain slices were stained for albumin by applying albumin antibodies (Dakopatts), by which albumin is revealed as brownish discolorations. Dark neurons were revealed by staining for RNA/DNA with cresyl violet.

In series of more than 1800 Fisher rats, we have proven that sub thermal power levels from both pulse-modulated and continuous RF fields – including those from real GSM mobile phones - have the potency to significantly open the BBB for the animals' own albumin (but not fibrinogen) to pass out into the brain and to accumulate in the neurons and glial cells surrounding the capillaries. Albumin extravasations are most prominent at the lower SAR values. This dose-response behaviour suggests some kind of energy or electromagnetic field strength windowing effect. A linear dose-response relationship for dark neurons was found at 50 days after exposure, with most prominent occurrence at SAR 200mW/kg.

Introduction

Since 1988 we have studied the permeability of BBB to endogenous albumin and fibrinogen during exposure to various electromagnetic fields. For more than 10 years we have studied the effects from exposure to electromagnetic fields used in GSM mobile communications on the rat brain and found increased permeability of the blood brain barrier (BBB) to endogenous albumin [1-4]. The BBB has the function to regulate transport of substances between the blood and the brain in mammals and is of utmost importance for the protection of the brain from harmful compounds. Pronounced leakage was seen at the lowest power depositions (SAR<2mW/kg), while at SAR values at 200 – 2000mW/kg the albumin leakage was less pronounced.

Opening of the BBB by mobile phone radiation has been confirmed by other researchers. In two studies albumin leakage was found after exposure of rats with microwaves both in thermal and non-thermal SAR regions [5,6]. One study exposing only 12 animals, with microwaves at high SAR (2000 mW/kg) reported no significant albumin leakage [7]. Our findings that low intensity GSM 900 MHz electromagnetic fields influence the BBB is also supported by other findings in the in-vitro proteomic studies on a human endothelial cell line [8,9]. Recent findings show that microwave radiation with SAR values of 15-20 mW/kg induces albumin denaturation and promote amyloid fibril formation [10]. Those findings are most relevant to explain the mechanism of protein transfer through the BBB at low SAR levels. Albumin is toxic to neurons and may furthermore, as a transport protein, carry other harmful molecules into the brain parenchyma. We have also studied albumin uptake into neurons and has recorded occurrence of damaged neurons as well [11]. This new knowledge about the effects of low level microwave exposure on the brain should be considered in the foundation of exposure limits that take into account non-thermal biological effects of microwaves radiation from mobile telephones and base stations.

Material and Methods

A transverse electromagnetic transmission line chamber (TEM-cell) used for the exposure of rats was designed by dimensional scaling to fit 915 MHz from previously constructed TEM-cells at the National Bureau of Standards [12]. The outer conductor is made of brass-net and is attached to the inner walls of a wooden box and the centre plate, or septum, is made of aluminium.

In each TEM-cell two animals were placed in Perspex boxes above and below the central plate. They were awake during the exposure and could move and turn around within the exposure chamber. At the end of exposure they were anaesthetized and sacrificed by perfusion-fixation with 4% formaldehyde. Each brain was sectioned coronally in 1-2 mm thick slices, which all were embedded in paraffin and cut at 5 micrometer. Albumin was demonstrated with the IgG fraction of rabbit anti rat albumin (Cappel Research Products, Organon Teknika, Västra Frölunda, Sweden) diluted 1:16 000. Biotinylated swine anti rabbit IgG was used as a secondary antibody. Then avidin, was peroxidase conjugated to the biotin and visualised with DAB (diaminobenzidine), counterstained with Meyer-HTX (Dakopatt AB, Älvsjö, Sweden). Albumin and fibrinogen leakage is revealed as brownish spotty or more diffuse discolorations. Cresyl violet staining of RNA/DNA was used to reveal *dark neurons* which are regarded as damaged and appear shrunken, densely and darkly stained neurons. The occurrence of blood-brain barrier leakage and damaged (dark) neurons in different parts of the brain is judged blindly in a semi-quantitative way by the neuropathologist by given ranking values of increasing degree of albumin leakage: 0 (for no leakage); 0.5; 1.0 (for weak leakage); 1.5; 2.0 (for moderate leakage); 2.5 and 3.0 (for severe leakage).

Results

The results of BBB-permeability of albumin in rats exposed to 915 MHz microwaves of continuous

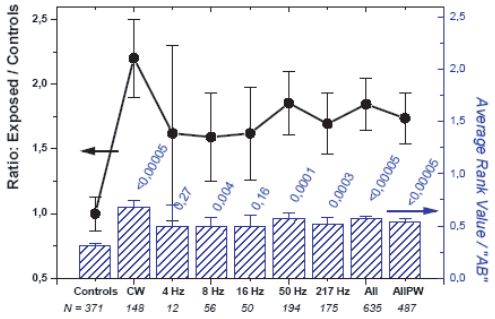

Figure 1: The histogram show the average rank values ("AB") for BBB albumin leakage in Fischer 344 rats exposed to 915 MHz microwaves modulated at various number of pulses per s (Hz). The p values represents the significance in difference from the controls. The upper line represent the ratios of the average rank values between exposed and controls. The modulation frequency and number of rats in each group are displayed below the axes.

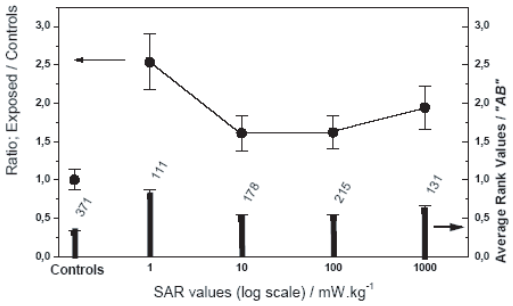

Figure 2: The histogram show the average rank values ("AB") for BBB albumin leakage in Fischer 344 rats exposed to 915MHz microwaves at SAR values. The number of rats in each group is displayed next to the bars. The upper line represents the ratio of the average rank values between exposed and controls.

wave and with different modulation frequencies at various SAR values ($4{\cdot}10^{-4}$ -$8{\cdot}$ 10^{-3}W/kg) are displayed in Fig.1 The average score values of albumin leakage in Fischer 344 rats (controls and exposed with 915 MHz microwaves at various modulation frequencies) are displayed in the histogram at the bottom of Fig.1 The ratio between exposed and controls shown by the line is larger than 1. There is no pronounced difference between the various modulations frequencies other than the effect of CW seems to be more effective in opening the BBB. This is surprising since the common opinion is that modulated microwaves would be more biologically effective. The histogram in Fig.2 shows the average rank values ("AB") for BBB albumin leakage in Fischer 344 rats exposed to 915 MHz microwaves at various SAR values. The number of rats in each group is displayed next to the bars. The upper line represents the ratio of the average rank values between exposed and controls. The SAR dependence is very similar to that previously reported by Oscar and Hawkins (1977) for the microwave alteration of the blood-brain barrier system of rats [13].

The appearance of dark neurons in a cresyl violet staining, clearly more frequent in exposed than unexposed animals, is used as an indicator of neuronal damage, both within the nucleus and the soma, in the long run

resulting in neuronal shrinkage. Albumin leakage was still more evident at the lower SAR-values (2mW/kg), but the occurrence of dark neurons was most significant at 20 and 200mW/kg [11]. This finding might indicate that different mechanisms might be responsible for albumin leakage and neuronal damage. We consider albumin leakage to be a sensitive and reproducible model for studying the interaction of electromagnetic fields with cell membranes. BBB-leakage is an active, vesicle mediated, transport through the blood vessels endothelial cells (pinocytosis) [14].

Relevance to Society

The possible risks by radiofrequency electromagnetic fields exposure of the human body, is a major concern for the society. A new, third generation of mobile communication is becoming increasingly important, but the health impact of this radiation modality is largely unknown [15]. Epidemiological studies will not be able to answer this question until after 10 – 15 years of exposure [16,17]. It is therefore of greatest importance to study in the laboratory biological effects that can lead to health impairment [18]. It is of great importance both to quantify the leakage of albumin through the BBB and to study the toxicological effects of this leakage. This new knowledge can be used as a foundation for new exposure limits that take into account non-thermal biological effects of microwaves radiation from mobile telephones and base stations.

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