First Results of a Long-Term Epidemiological Study on Low-Level Microwave Exposure of Rats

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***Abstract* — The general public applications of microwaves are essentially the cellular phone and the microwave oven. Some years ago, articles in the national and international press started to express concern about cancer incidences among radar technicians and people housed in the neighborhood of antenna base stations. In this study, a possible link between microwave exposure and the physiological and cellular changes is being evaluated by an epidemiological long-term animal study on 120 rats, for 21 months. In order to assess the possible biological effects of microwaves, we selected among others the following parameters: lymphocytes, monocytes, granulocytes, erythrocytes and blood platelets.**

***Index Terms* — Biological cells, biological effects of radiation, blood, CW radar, military communications, radar.**

I. Introduction

In March 2004, we started an epidemiological study [1] on the possible biological effects of microwaves, because of the current importance of this topic in many European countries. The main impulse for this study came from the Belgian Minister of Defense. It was set up due to his concern regarding the cancer incidences among military personnel that has been working with the radar system of the HAWK air defense system. Besides this military relevance of the study, there is also public interest because of the possible biological effects of exposure coming from the cellular phone handset and the base stations. Very few people are exposed to thermally significant levels of microwaves. The great majority of exposures occur at levels at which weak-field interactions would be the only possible source of any adverse health response. The lack of consensus regarding the low-thermal long-term effects of microwaves pushed the WHO (World Health Organization) to state that more scientific research is needed [2]. Our research must be considered in this framework.

II. Objectives of the Study

The present study is a long-term epidemiological study on rats, aiming to trace possible physiological and cellular changes in 120 *Wistar* albino rats due to low-thermal microwave exposure at a frequency of 1 GHz and 10 GHz, pulsed and continuous wave (CW), respectively. This paper is mainly focused on the performance evaluation of the immune system and histological post-mortem analysis together with a possible stress induced response. Biological endpoints are among others different types of white blood cells, namely lymphocytes, monocytes, eosinophyls, basophyls and neutrophyls, erythrocytes, blood platelets, cytokines and stress induced hormones as ACTH (adrenocorticotrophic hormone) and corticosterone. Body weight is monitored too, because it appears to be a reasonably sensitive indicator of stress.

III. An Innovating Concept in Animal Epidemiological Research

In the experiments three-month old male *Wistar* albino rats were collectively exposed in a spacious exposure unit. In most of the other studies, rats are kept separately during exposure, where space to move is limited. Findings in animal psychology research learn that narrow housing causes an enormous stress response in the rat [3]. This stressor creates a supplementary variable, which is susceptible to mask secretion of certain corticosteroids [4]. Besides, observing freely moving animals gives supplementary information on the influence of exposure on behavior and locomotor activity as an indicator of an effect on the central nervous system.

IV. Microwave Exposure System

The entire microwave exposure system is composed of four exposure units, each for one exposure type (i.e. 1 GHz pulsed, 1 GHz continuous wave, 10 GHz continuous wave) together with the sham-exposed group.

The exposure unit is a self-made cage, suitable for 30 freely moving male rats, with an antenna on top. Its bases consists of a custom made box of polyethylene (1.11 m x 0.60 m x .71m) covered at the outside with radar absorbing material, itself covered with wood (multiplex 15 mm) to prevent any artefacts arising from objects placed in the microwave field. The cover of the box is also self-constructed. The height is such that the rats are exposed in the far field of the antenna. The 1 GHz continuous wave exposure unit is equipped with a Kathrein GSM antenna (0.325 m x 0.265 m x 0.05 m) characterized by a gain of 9 dB and a 3-dB beamwidth in the horizontal and vertical plane of 65° and 70°, respectively. A second group of 30 rats is exposed to a 1 GHz pulse-amplitude-modulated signal, with a pulse repetition frequency of 1 Hz and a duty

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cycle of 10%. The antenna is an of-the-shelf Kathrein GSM antenna, vertical polarization with a 9 dB gain; the half power beams in the E-plane and the H-plane are 65° and 70°, respectively. For the 10 GHz CW exposed group, we used two X-band 16-dB horn antennas (0.0725 m x 0.05m) which are integrated with an interdistance of 0.5 m in the cover of the exposure unit. Both identical antennas have a 3-dB beamwidth of 28° and 30° in the E-plane and the H-plane, respectively. The last group of 30 rats is sham exposed: they enter every day in an identical exposure unit, but the only different variable is the absence of exposure. In each of the exposure unit the E-vector is orientated parallel to the long axis of the rats' body.

Fig. 1. Collective exposure system for freely moving rats.

We have set up a protocol suited for evaluating the possible biological effects of low-thermal microwave exposure in rats. The resolved mean exposure level in absence of the rats is respectively 200 µW/cm² for the 1 GHz exposure and 500 µW/cm² for the 10 GHz exposure. These exposure levels are derived from the ICNIRP recommendations [5], based on the thermal effects of microwaves on the human body. Assuming that the ratio in size between a human and a rat equals 10, we adapt the ICNIRP reference levels, valuable for a human being, to the size of the rat by multiplying by 10 the key frequencies. This means that the exposure of a rat of 1 GHz corresponds to a human exposure of 100 MHz. The basis ICNIRP reference value for man at 100 MHz is 2W/m². This means that a power density of 200 µW/cm² is suitable for the rats at 1 GHz. Following an identical reasoning leads us to the fact that a 1 GHz exposure for man corresponds to a 10 GHz exposure for rats. ICNIRP recommendations stipulate a reference level for man, expressed in power density, of f/200 (f = frequency), i.e. 1000 MHz/200 or 5 W/m² or 500 µW/cm². This exposure level is chosen for the rats at 10 GHz. The corresponding SAR value is 0.08 W/kg and is to be considered as low-thermal: the ICNIRP basic restrictions are based on acceptable thermal effects, below which no adverse effects occur.

A well-established dosimetry of the incoming electric field in nearly 80 sampling points inside each unit was carried out before the start of the experiment. The mean value of the recorded power density in the 1 GHz and the 10 GHz exposure unit is 206 µW/cm² and 320 µW/cm², respectively.

The rats are exposed in this exposure system (Fig. 1) two hours a day, seven days a week during one and a half year, since 15 March 2004.

V. The immune system as a key factor in the cancer pathogenesis

The immune system plays a crucial role in the upholding of the general health condition. This is the reason why we have chosen in the first place for a quantitative study of leucocytes and cytokines as a possible biological indicator of a decreasing health status. A failing immune system can give birth to tumor cell proliferation processes. Indeed, the immune system is of great importance in defense against bacteria, intracellular pathogens, parasites and cancer cells or neoplasms; it is a primary determinant of survival. The immune system consists of cells that are specialized for defense broadly classified into phagocytic cells and lymphoid cells, and cell-derived humoral substances such as antibodies and complement. The latter factors can attach to challenging organisms and lyse them directly, or serve as handles to enhance the ability of phagocytic leukocytes to attach via receptors, and phagocytose and kill organisms. Cell-mediated immune mechanisms are the major defenses raised against intracellular pathogens and neoplasms. Cell-mediated immunity is established by lymphocytes, and monocytes or monocyte-derived tissue macrophages. Humoral immunity and phagocytic cells (especially neutrophyls) are of paramount importance in defense against bacterial species and other microbes.

Cytokines are small secreted proteins which mediate and regulate immunity, inflammation and hematopoiesis. They generally act over short distances and short time spans and at very low concentration. Most cytokines activate other cells of the immune system or regulate cell death of tumor cells.

VI. Blood sampling, data collection and statistical analysis

During the duration of exposure, blood samplings have been periodically taken every three months on each rat. We selected 15 physiological parameters to be analysed in each blood sample of the 120 rats. In total we have to analyse nearly 10 000 individual rat data.

In the first place, a selection of pertinent parameters for further detailed analysis has to be carried out to reduce the complexity of the task. Following this reasoning, we compared variables from exposed and sham exposed rats and made a selection of these parameters which showed major differences between exposed and sham exposed groups. To avoid problems of multiple tests, we decided on the non parametrical statistical approach of Significant Analysis of Microarray (SAM) [6] based on permutations, which compares the values of all parameters from the sham exposed groups with all parameters from the exposed groups during the entire 18-month exposure period, trying to select those which differ significantly. This operation ends up with a list of 15 parameters containing those parameters presenting possibly a major difference between exposed and non-exposed groups for the subsequent bloodsampling sessions. The false discovery rate regarding the whole list is 25%, while the possibility of error for each parameter on the list is 5%. Since the design of the study is such that the only distinction between those two groups of rats (exposed versus non-exposed) is the exposure, we elaborate the statistical analysis on those 15 detained parameters by an ANOVA test (Analysis of Variance between groups). The ANOVA specifies if there is a statistical significant difference ($p < 5\%$) between at least 2 of the 4 groups for that specific parameter.

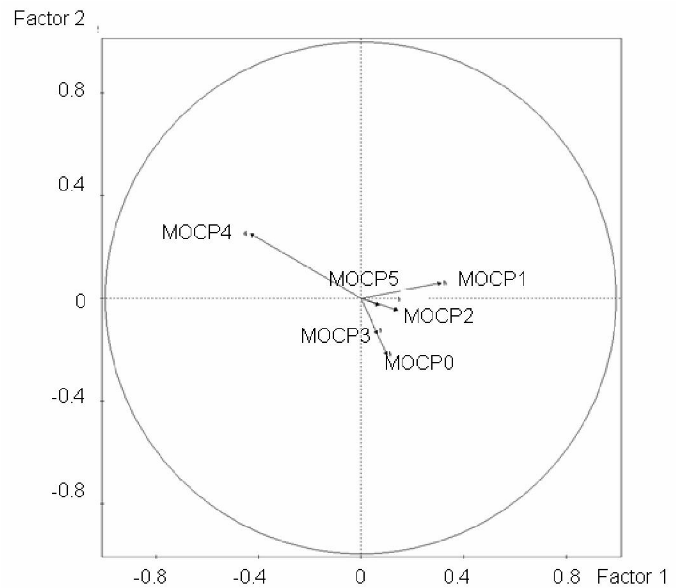

Fig. 2. Correlation circle regarding monocytes (MOC) during the subsequent blood sampling periods (P0 to P5).

We proceed with a descriptive analysis (boxplot) to target the groups susceptible to explain the founded statistical difference. Finally a t-student test compares the values of the selected parameter with those of the sham exposed group. The statistical method of Principal Component Analysis (PCA) is a suitable tool to confirm didactically earlier findings. The PCA is a multidimensional method suitable for describing a correlation between variables. PCA transforms the original variables into non correlated independent variables, called principal components, a linear combination of the original variables allowing to represent each parameter by one single value. The first two principal components define a plane for which the variance in the projection is maximal and where the different parameters are illustrated by a dot. With two factors we can explain most of the variance of the observed data. Passing from a multidimensional plane to a two-dimension plane affords data which are easier to approach. When the variables are close to the limit-circle their projection to the plane is very good. When two variables are highly correlated their representations are very close to one another on the circle. On the other hand, when two variables are negatively correlated their representations are on opposite sites (e.g. MOCP2 and MOCP4). When two variables are not correlated, their representations are perpendicular. This can be observed in Fig. 2, where MOC stands for monocyte and the suffix is each time related to the respective blood sampling period, starting from P0 to P5, P0 being the reference blood sampling. Indeed, MOCP0 and MOCP1 are nearly perpendicular to each other, so they are very little correlated: there is nearly no association between the monocytes before exposure and after a 3-month exposure period. This finding could indicate a statistical significant difference between MOCP0 and MOCP1. This hypothesis will be tested in chapter VII.

The correlation circle is a graphic representation of the correlation matrix and of the correlations between the initial variables and the principal components.

VII. First Results

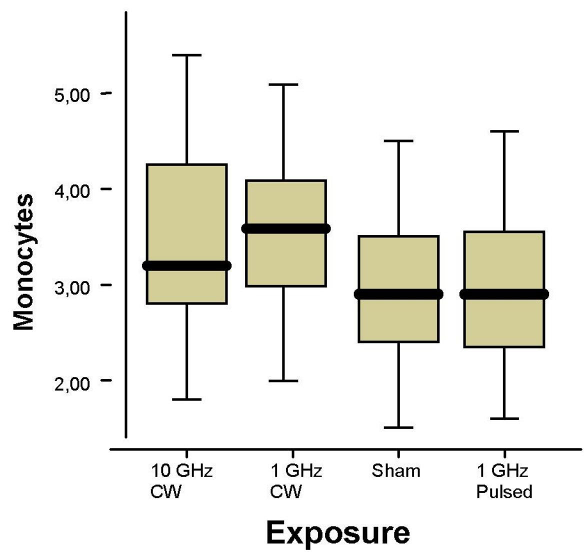

Fig. 3. Boxplot of monocytes for the different exposure types.

After a three-month period of exposure, an effect of the 1 GHz CW exposure on the monocytes showed up using the ANOVA test accepting a threshold of 5% (confidence interval 95%). A calculated p-value of 0.002 indicates there is a statistical significant difference between at least 2 of our four groups of rats for this parameter. The descriptive analysis of this finding by means of a corresponding boxplot (Fig. 3) confirms that there is a difference between the 1 GHz CW exposure and the sham exposure. An independent t-student test concludes with the existence of a statistical significant difference ($p < 5\%$) between the 1 GHz CW exposed group of rats and the sham exposed group, showing a p-value of 0.003.

VIII. Conclusions

In the present stage of the statistical analysis, we have focused on the effects of microwave radiation on the basic cells of the immune system. On the basis of this objective approach for selecting a target parameter explained in paragraph IV, we have chosen in a first time the monocytes for further analysis.

We exposed four-month-old *Wistar* albino rats during 21 months to two different frequencies and exposure modes. After 3 months of exposure – two hours a day, seven days a week – we found a statistically significant difference ($p = 0.003$) between the 1 GHz CW group and the sham exposed group regarding the monocytes. Monocytes are the largest of the white blood cells and play an important role in the good functioning of the immune system. The exposed group showed an increase in monocytes percentage of 20% compared to the sham exposed group. Probably the exposure has influenced the immune system and it has reacted to this stressor by increasing the percentage of monocytes in the peripheral blood circulation. Further research related to the biological relevance of this finding is needed.

A sub entity of the present epidemiological study deals both with a mortality study and a cancer study. The mortality rate is indeed a good indicator of the overall health status of the animals in the different groups. Further on, the cancer incidence in all groups of exposed rats will be compared to the cancer incidence in the sham exposed group. Preliminary results are expected within three and a half months.

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