LOW FREQUENCY LOW INTENSITY PULSE ELECTROMAGNETIC FILED *IN VIVO* INFLUENCE ON IMMUNE CAPACITY IN RAT

CĂLIN LUCIAN MANIU*, LUCIAN HRIŢCU[1]

Keywords: pulse electromagnetic field (PEMF), in vivo, leukocytes, antibody titer, albumin/globulin ratio, rat.
Abstract: The *in vivo* influence of a low frequency and low intensity (50 Hz, 2.7 mT) pulse electromagnetic field (PEMF) applied continuously 30 min for a period of 20 days, upon the immune reactivity in rats was studied. For this aim, leukocytes, antibody titer, the albumin/globulin ratio were determined by appropriate methods in rats treated with PEMF under normal conditions, under stress conditions of isolation and under sulpiride treatment as dopamine D2 receptor blocking agent. Isolation stress induced an increase of leukocytes total number and a leucocyte formula modification, which indicates an attempt to increase the cellular mediated body defense capacity of the stressed organism. Also, the stress of isolation reduces the amount of antibody, which means a possible reduction of humoral mediated defense capability. However, under stress of isolation an increase of albumin/globulin ratio values were observed. PEMF treatment induces a recovery trend of antibody titer and leucocyte formula values. PEMF combined with sulpiride and isolation stress treatment determined recovery of albumin/globulin ratio, indicating an antistress effect of PEMF treatment, which is achieved probably through D2-dopamine receptor.

INTRODUCTION

In the past twenty years, research on biological effects of electromagnetic fields, not only have expanded, they became a subject of public concern and debate throughout the world. Generally, fields and electromagnetic waves interaction with biological systems is closely related to the frequency, mechanisms of interaction of low frequency fields are very different from those of high frequency. At low frequencies, the electric and magnetic components of an electro-magnetic field are independent, which means that it isn't a true electro-magnetic field as it happens at much higher frequencies (Chang & Wait, 1974, Olsen & Wong, 1992). In practice, very low frequency electromagnetic fields are considered as domain 0-300Hz (Lambrozo, 2001). While significant progress is made on several fronts, a special emphasis is increasingly directed toward understanding the most comprehensive details of the effects of electromagnetic fields of low and very low frequency.

Over the past decade there has been a growing interest in the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on bacteria among researchers in this field. Most of the studies in this area have shown that ELF-EMF in the range of 50 Hz, 1–10mT and applied for 1–24 h causes a decrease in the growth rate of bacteria (Strasak et al., 2002; Fojt et al., 2004; El-Sayed et al., 2006). The effect of ELF-EMF on the phagocytic cells of the immune system has attracted much attention due to their crucial role in cancer. Further, an important aspect of these phagocytic cells is that they produce high levels of free radicals in response to infection, and the effect of ELF-EMF on free radicals has been proposed as a probable mechanism for the effect of ELF-EMF on living systems (Brocklehurst & McLauchlan, 1996; Simko, 2004). Accordingly, it is inevitable that ELF-EMF will affect phagocytic cells. Also, several studies have shown the effect of ELF-EMF on macrophages. Macrophages, which have a crucial role in innate immunity, are activated by the binding of pathogens or by local cytokine release. It was showed that EMF exposure decreased viability of J774.2 macrophages (Kawczyk-Krupka et al., 2002). Increased free radical levels were detected in human monocytes and mouse macrophages after exposure to 50 Hz, 1mT ELF-EMF (Simko et al., 2001; Lupke et al., 2004; Rollwitz et al., 2004).

Apoptosis of host cells has been shown to be an important step for the modulation of pathogenesis. In order to evade death resulting from exposure to free radicals, pathogens have evolved mechanisms, one of which is the modulation of apoptosis of the host cell. While some pathogens block apoptosis of host cells, others induce apoptosis to defend themselves against the host (Gao & Kwaik, 2000; DeLeo, 2004). Heat shock proteins (HSP) are evolutionarily conserved proteins known to play a key role in cellular defense against the effect of stressors, and their function in modulating apoptosis has been well assessed (Creagh et al., 2000; Beere, 2004). Goodman et al. (1994) first demonstrated that HSP expression was enhanced by exposure to electromagnetic fields. Tokalov and Gutzeit (2004) showed the effect of ELF-EMF on heat shock genes and demonstrated that even a low dose of ELF-EMF (10 mT) caused an increase in HSPs, especially hsp70, implying that the cell senses ELF-EMF as a stressor.

However, these very low frequency fields interact very differently in relation to the tissues diversity and physiological processes occurring in living systems. Also, it must bear in mind the negative effect of the electromagnetic field impact on the living structures and cellular processes with highly sensitivity to this physical phenomenon. Therefore, the

positive effects of some kind of radiation with a certain frequency and intensity that occurs strictly localized, is not a guarantee of a general positive biocompatibility, fact which requires a greater and larger study of biological effects. Given these issues, it was considered useful to investigate, in every way possible, the influence of a low frequency and intensity electromagnetic fields with the normal (healthy) organisms often facing in nature, as well as highlighting the potential biological effects of biomedical applications. Therefore, in the experiments was used a device that is used for medical purposes.

MATERIALS AND METHODS

A MAGNETODIAFLUX device that generates a pulse electromagnetic field (PEMF) was used for treatment. Irradiation is accomplished by means of two Helmholtz coils connected to the device. If using two Helmholtz coils, the ideal solution to have a uniform electromagnetic field is their arrangement at a distance equal to their radius (Crowell, 2010). MAGNETODIAFLUX device feeds the two coils with a pulsating direct current (PDC) obtained by converting and rectifying the 220V/50Hz AC power. The PDC has a frequency of 50Hz or 100Hz, the PDC peak voltage variation indicated on the oscilloscope is 42V with 0.7A at 50Hz, respectively 1.4A at 100 Hz. The coils are 29cm in diameter with approximately 630 turns. To obtain a uniform field, two coils were placed at a distance of 14.5 cm. Based on the known physical values it was calculated the magnetic flux density on the central axis. Resulting values are 2.7 mT for 50Hz and 5.5 mT for 100Hz PDC. The two values were also verified by a direct measurement of the magnetic flux density using a digital tesla-meter for safety and accuracy of the experiment. As experimental animals were used white Wistar rats divided into homogeneous groups as sex, age and weight. In this experiment it was used a 2.7mT PEMF. Stress factor was the introduction of animals used individually in Plexiglas tubes of 1.5L. This tube isolation model is a very powerful form of stress for animals undergoing the experiment. Before carrying out the tube stress session isolation, rats were handled individually for a month, 10 minutes daily to remove any form of stress induced by contact with humans. The animals were placed individually in Plexiglas tubes of 1.5L, equipped with multiple holes to allow ventilation. The animals were subjected to stress sessions daily for 20 minutes, during 18 consecutive days without access to food and water.

The group of animals under tube stress action was accompanied by a control group consisting of animals that were kept in the cage maintenance with access to food and water. After the experiment were transferred to the cage maintenance. At 18 days after application the tube isolation stress, the animals were killed, the blood collected for determining immune indices. More, it was pursued the involvement of dopamine D2 receptors in immune regulation, it was used sulpiride (Sulp) (Sigma) (4 mg/kg b.w.), a specific antagonist of D2 dopamine receptors, administered daily for 18 consecutive days. To this end, it was used the following groups of animals: LOT1, the neutral control group: i.p. immunization with a lipopolysaccharide solution (LPS) from *Escherichia coli*, serotype 0111: B4, Sigma (25µg/25µl) administered in a volume of 0.1 mL/100g/rat in the first day of the experiment. LOT2: i.p. immunization with a solution of LPS (25µg/25µl) administered in a volume of 0.1 mL/100g/rat in the first day of the experiment. At 3 days after immunization, were given daily i.p. sterile saline solution 30 minutes prior to tube stress procedure. The tube isolation stress was used 20 minutes daily for a period of 18 consecutive days from the onset of the experiment. LOT3: i.p. immunization with a solution of LPS (25µg/25µl) administered in a volume of 0.1 mL/100g/rat in the first day of the experiment. At 3 days after immunization, were given daily i.p. sulpiride solution (4 mg/kg b.w.) 30 minutes prior to tube stress procedure. The tube isolation stress was made 20 minutes daily for 18 consecutive days from the onset of the experiment. LOT4: i.p. immunization with a solution of LPS (25µg/25µl) administered in a volume of 0.1 mL/100g/rat in the first day of the experiment. At 3 days after immunization, were given daily i.p. sterile saline solution, 30 minutes prior to tube stress procedure. The tube isolation stress was used 20 minutes daily for 18 consecutive days from the onset of the experiment. At 3 days after LPS administration, rats were subjected to PEMF treatment 20 min. each day for 18 days. LOT5: i.p. immunization with a solution of LPS (25µg/25µl) administered in a volume of 0.1 mL/100g/rat in the first day of the experiment. At 3 days after immunization, were given daily i.p. sulpiride solution (4 mg/kg b.w.) 30 minutes prior to tube stress procedure. The tube isolation stress was applied 20 minutes daily for 18 consecutive days from the onset of the experiment. At 3 days after LPS administration, rats were subjected to PEMF treatment 20 min. each day for 18 days.

RESULTS AND DISCUSSIONS

From the results on the total number of leukocytes are found that tube isolation stress causes a significant increase in the number of leukocytes of isolated animals compared to control group. This response to stress conditions is indicating an attempt to increase the defense capacity of the body under stress. The group of animals subjected to stress where D2-dopamine receptors,

involved in immune regulation, were blocked by sulpiride, reveals a reduction in the number of total leukocytes, significant than the level recorded in conditions of stress, which implies a reduction in defense capability (Fig.1).

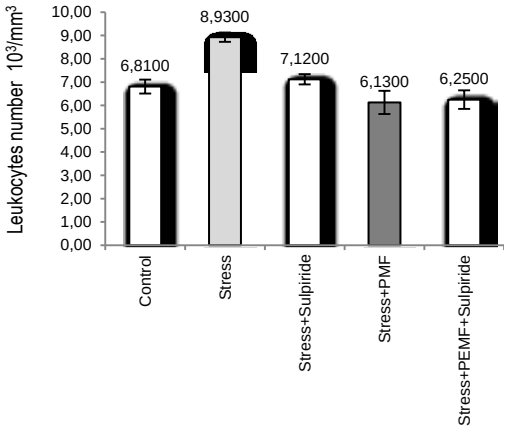

Fig.1. The change of leukocytes number after 18 days of rat immunization, under sulpiride and/or 50 Hz, 2.7 mT PEMF treatment, applied 30min/day.

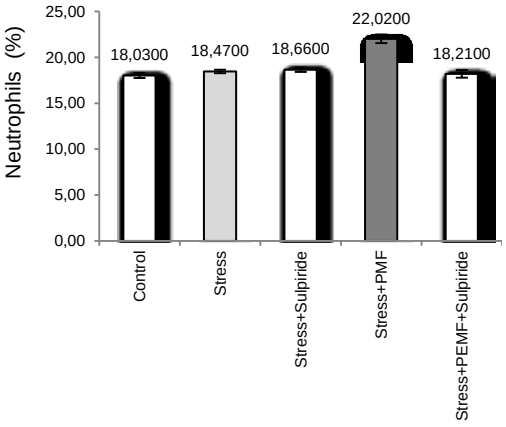

Fig.2. The change of neutrophils percentage after 18 days of rat immunization, under sulpiride and/or 50 Hz, 2.7 mT PEMF treatment, applied 30min/day.

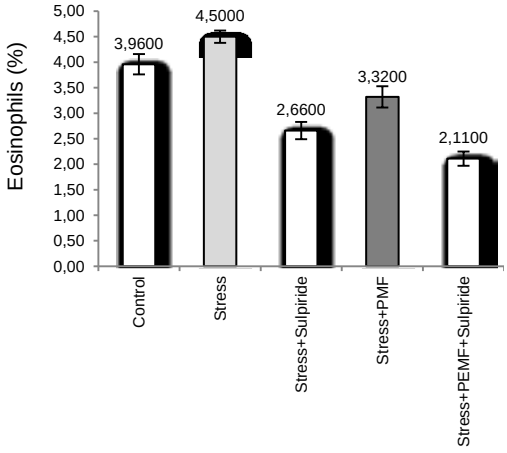

Fig.3. The change of eosinophils percentage after 18 days of rat immunization, under sulpiride and/or 50 Hz, 2.7 mT PEMF treatment, applied 30min/day.

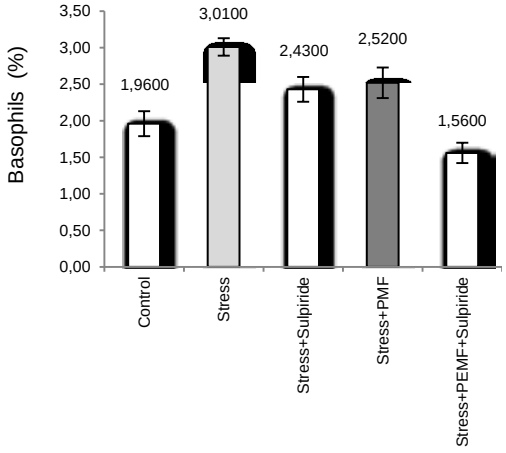

Fig.4. The change of basophils percentage after 18 days of rat immunization, under sulpiride and/or 50 Hz, 2.7 mT PEMF treatment, applied 30min/day.

Regarding leukocyte formula reactivity, there is a more diverse response with specific aspects of each leukocyte group (Fig.2-5). Thus, regarding the neutrophils, there is a clear effect only in the case of PEMF action under stressed animals, expressed by a significant increase in neutrophils, the other experimental variants giving no changes in values compared with the control, thus it is not realizing a phagocytic capacity enhancement (Fig.2).

Isolation of animals leads in both cases (eosinophils and basophils), an increase in leukocyte number of these groups as a reaction to stress conditions, but this effect was canceled by concomitant treatment with sulpiride (Fig.3-4). PEMF singular stress effect on animals is generally similar to that of sulpiride. When the PEMF is applied simultaneously with sulpiride, produce a sharp reduction in leukocyte number of these groups, which implies an expansion of the action one by the other agents. Under these conditions, treatment with PEMF does not seem to have beneficial effects for the body.

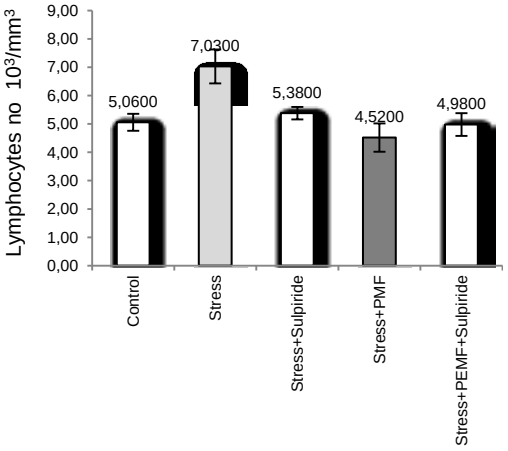

Fig.5. The change of lymphocytes number after 18 days of rat immunization, under sulpiride and/or 50 Hz, 2.7 mT PEMF treatment, applied 30min/day.

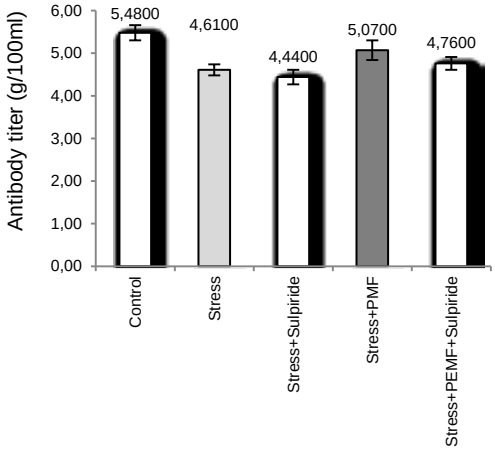

Fig.6. The effect of sulpiride and/or 50Hz, 2.7 mT PEMF treatment applied 30min/day on the percentage of antibody titer in the 18th day after immunization, under the stress conditions.

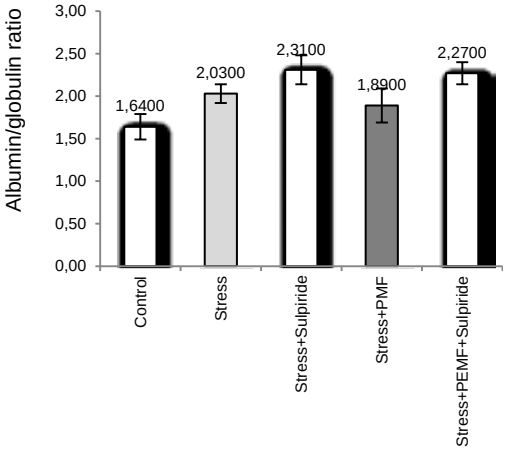

Fig.7. The effect of sulpiride and/or 50Hz, 2.7 mT PEMF treatment applied 30min/day on albumin/globulin ratio in the 18th day after immunization, under the stress conditions.

Regarding the proportion of lymphocytes (Fig.5), the situation is very similar to those in the total number of leukocytes, which is likely, given their values in the same order of magnitude. It is recorded as a gain in the number of lymphocytes due to the tube isolation stress, which is stopped by treatments applied in different versions, as with total leukocytes.

Aspects of attention are observed in antibody titer (Fig.6). Stress isolation conditions induce a small amplitude reduction of values, but significant, implying a decrease, in these conditions, of the defense capacity humoral mediated. Blocking with sulpiride of D2-dopamine receptors at the stressed animals, is not leading to the recovery of antibody titer values. PEMF treatment allows a return to normal antibodies, although there is not a numerical evident increase in lymphocytes. A similar

effect is also found in the sulpiride and PEMF simultaneously treatment, assuming a cancel of the blocking effect of sulpiride.
From values of the albumin/globulin ratio (Fig.7) that isolation stress induces a significant increase compared to control. This effect is attenuated by only PEMF treatment when it is recorded a back to normal trend. The sulpiride treatment of stressed animals is recorded, however, a trend of increasing the values of this ratio in both singular and combined with PEMF sulpiride treatment. Such variations may be based on value changes induced by treatment, either of the proportion of albumin, or on that of globulin, which implies a correlation with the effects of applied treatments on defense capability.

CONCLUSIONS

While the animals under stress were treated with 50Hz/2.7 mT PEMF, the field action is manifested also by a reduction in the number of total leukocytes, compared with their level recorded from tube isolated animals without any treatment, without a big difference from the control group values. Such an effect, similar to that of sulpiride, could result, in these conditions, probably from an inhibitory action on D2-dopamine receptors. To elucidate this issue are necessary further investigations. When PEMF acts simultaneously with sulpiride on stressed animals, there is no difference to single action of the two agents. This can be explained by a competition of the two agents in terms of their specific place of action, entire organism reactivity does not occur in a summation of their specific effects.
Isolation stress causes a significant increase the albumin/globulin, compared with the control, but this effect was diminished by treatment with PEMF applied singular. The combined PEMF with sulpiride treatment as D2-dopamine receptor blocker and with isolation stress, there is a certain recovery by an increasing albumin / globulin ratio values, which indicates a possible increase in immune reactivity.
These results reflect a possible anti-stress effect of PEMF treatment. This effect is similar to that seen in animals with tube isolation stress treated with sulpiride, a D2-dopamine receptor blocking agent, indicating the possibility to achieve the anti-stress PEMF effect via these D2 receptors.
PEMF stimulating effects on the leukocyte groups could be useful in some medical applications (infection, inflammation and allergies).

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[1] University „Alexandru Ioan Cuza" Iaşi, Romania, Faculty of Biology.
Address: Carol I avenue, no. 20 A, Iaşi, Romania, code 700505; tel. +40(232)201500;
* e-mail: cmaniu@uaic.ro