

Effects of pulsed magnetic field chronic exposure on some hematological parameters in mice

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SUMMARY

In this experimental study, the hematological effects of pulsed electromagnetic fields (EMF) chronic exposure were investigated on mice. Sixty, 6 week old male Swiss mice, weighing 40-45g were used, and were divided into two groups: in one group, animals (n=30) were exposed to pulsed EMF (60 Hz, intensity 3μT, twelve hours by day) for a 120 day-period, whereas the second group (n=30) was used as control. On days 15, 30, 90 and 120, samples were taken by cardiac puncture for the hematological analysis (Red blood cell and white blood cell counts, leukocyte distribution). Whereas no significant difference was noted between control and exposed animals at the 15th and the 30th days, a macrocytic anemia characterized by decreases of hemoglobin concentration, hematocrit values and erythrocyte counts and by the increase of mean corpuscular volume, occurred in the exposed animals on day 90. Furthermore, they have shown significant reductions of leukocyte, lymphocyte and neutrophil counts, while monocyte counts were increased. On day 120, these leukocyte alterations were still observed, whereas erythrocyte parameters became close to control values.

These results suggest that pulsed electromagnetic fields (60 Hz and 3μT) affect the hematological parameters of mice, probably by reducing proliferation and differentiation of marrow stem cells.

Keywords : Pulsed magnetic field - hematological parameters - mice.

RÉSUMÉ

Effets d'une exposition chronique à des champs magnétiques pulsés sur quelques paramètres hématologiques chez la souris. Par N. ÇETIN, A. BILGILI et G. ERASLAN.

Au cours de cette étude, les effets hématologiques d'une exposition chronique à des champs électromagnétiques pulsés (EMF) ont été étudiés chez la souris. Soixante souris suisses mâles, âgés de 6 semaines, et pesant 40-45 g ont été réparties en 2 groupes : dans un groupe, les animaux (n=30) ont été exposés à un champ électromagnétique pulsé (60 Hz, d'intensité 3μT, 12 heures par jour) pendant une durée de 120 jours, tandis que le 2^{ème} groupe (n=30) a servi de groupe contrôle. En vue d'une analyse hématologique les échantillons ont été prélevés par ponction cardiaque les 15^{ème}, 30^{ème}, 90^{ème} et 120^{ème} jours.

Alors qu'aucune différence significative n'a été observée aux 15^{ème} et 30^{ème} jours entre les animaux contrôles et exposés, une anémie macrocytaire caractérisée par une diminution de l'hémoglobine, de l'hématocrite et de la numération érythrocytaire et par une augmentation du volume corpusculaire moyen, est apparue chez les animaux exposés au 90^{ème} jour. De plus, ils ont présenté une leucopénie significative associée à une neutropénie et une lymphopénie, tandis que le nombre de monocytes a augmenté. Au 120^{ème} jour, les altérations leucocytaires ont encore été observées alors que les paramètres érythrocytaires sont devenus proches des valeurs contrôles.

Ces résultats suggèrent que les champs électromagnétiques pulsés (60 hertz et 3μT) affectent les paramètres hématologiques des souris, probablement en diminuant la prolifération et la différenciation des cellules de la moelle osseuse.

Mots-clés : Champ magnétique pulsé, paramètres hématologiques, souris.

Introduction

The search for biological effects of electromagnetic fields (EMF) exposure has been going on at an accelerating pace for more than three decades. However, the results of epidemiologic studies on the consequences of residential and occupational exposure with particular reference to cancer risk have been extremely controversial [7, 11, 13] because of methodological pitfalls and limitations. On the other hand, most investigations on carcinogenesis and mutagenesis using laboratory animals have been carried out under experimental field exposure conditions. An important issue of world wide concern and debate is that of a possible association between prolonged exposure to EMF field and cancer. Epidemiological studies have shown a positive correlation between environmental EMF and cancer [2, 8, 10]. Such EMF fields may affect the hematological parameters and therefore play a role in cancer promotion.

Some studies have investigated the effects of EMF on hematologic variables, but the results have been inconsistent.

Some researchers [1, 5] reported that EMF affected various hematological parameters such as neutrophil and lymphocyte counts, while some others [12, 16] have underlined that EMF have no effect on hematologic parameters. Consequently, this study was undertaken to evaluate the effects of 60 Hz fields on some hematologic variables in mice.

Materials and Methods

ANIMALS

Sixty, 6 week old male adult Swiss mice, weighing 40-45 g were used. Water and food were supplied *ad libitum*. Animals were housed in a vivarium with a 12-h light/ dark cycle, at temperature of 22 ± 1°C, in cages of dimensions 14x29x20 cm³. The cages were constructed with plexiglas to avoid interference with the magnetic field. A total of 60 mice were divided into two groups (control and exposed) of 30 animals in each group. The exposed group was housed in

five regular cages (six animals per cage) on the table. After adaptation to the laboratory environment for 2 weeks, animals were exposed to 60 Hz and 3 μ T field for twelve hours a day (7:00 h to 19:00 h) for 120 consecutive days ; simultaneously, control group were kept in remaining four cages in identical conditions as the experimental animals, except that the coils were not energized. Intensities of electromagnetic field were measured with a digital teslameter.

MAGNETIC FIELD EXPOSURE SYSTEM

Electromagnetic field used in this study is pulsed electromagnetic field. Generator of pulsed electromagnetic field is consist of two separate blocks. Working frequency of system can be adjusted between 20-100 Hz. System can provide a field strength of 1- 5 mT. The pulse-width of the output signal can be set between 0,2 and 2 milliseconds. The electronic circuit wiring has been designed so as to have the capability of driving 5 identical coils any successive pair of which may be regarded as a Helmholtz system. The each coil consists of 227 turns of 0.85 mm ϕ enameled copper wire. The coils have a diameter of 28 cm. and are separated by 14 cm.

In order to get the same field strength within the cylindrical volume formed the coils, the distance between the coils was selected to be equal to the radius of the coils [14]. Darlington transistors supplies 200 W and 20 A current needed for coils.

HEMATOLOGICAL ANALYSIS

Blood samples (0.5 ml per animal) were taken by cardiac puncture under light ether anesthesia on days 15, 30, 90 and 120 of study from both experimental and control groups. Blood samples were collected in K₃-EDTA tubes. Total erythrocyte (RBC), leukocyte (WBC) and platelet counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), hematocrit value, and percentage of neutrophil, lymphocyte, monocyte, eosinophil and basophil were determined using a blood counter (Symex SE-9000).

STATISTICAL ANALYSIS

Analysis of differences between exposed animals and controls were done using the Mann-Whitney U test. Statistical analyses were performed with the SPSS statistical package program for Windows. All data are presented as mean \pm standard deviation (SD). P<0.05 denoted a significant difference.

Results

Hematological parameters were listed in Table I.

In the control mice, red blood cell counts, mean corpuscular volume and mean corpuscular hemoglobin were slightly (but not significantly) increased on day 120, whereas hemoglobin concentrations were decreased. Also, platelet, total leukocyte, neutrophil and eosinophil (at a lesser extend) counts gradually increased according to time in the controls.

Parameters	Day 15		Day 30		Day 90		Day 120	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Hematocrit l/l	0.41 \pm 0.06	0.40 \pm 0.10	0.40 \pm 0.07	0.39 \pm 0.07	0.40 \pm 0.07	0.38 \pm 0.07*	0.41 \pm 0.07	0.42 \pm 0.07
Hemoglobin g/l	140 \pm 17	137 \pm 13	142 \pm 19	140 \pm 14	142 \pm 19	115 \pm 14**	130 \pm 16	139 \pm 15
Erythrocyte $\times 10^{12}/l$	9.0 \pm 0.5	8.2 \pm 2.2	9.3 \pm 0.6	9.0 \pm 0.7	9.3 \pm 0.6	7.5 \pm 0.7*	9.8 \pm 0.5	10.1 \pm 0.7
MCV fl	43.0 \pm 4.6	42.3 \pm 8.7	44.1 \pm 4.8	43 \pm 5.1	44.1 \pm 4.8	47.9 \pm 5.1*	46.9 \pm 6.1	48.1 \pm 7.1
MCH pg	18.8 \pm 1.3	20.2 \pm 2.1	17.9 \pm 1.2	18.2 \pm 1.5	17.9 \pm 1.2	18.2 \pm 1.5	19.2 \pm 1.6	18.9 \pm 1.5
MCHC g/l	353 \pm 78	341 \pm 67	373 \pm 81	367 \pm 79	373 \pm 81	367 \pm 79	357 \pm 75	375 \pm 62
Leukocyte $\times 10^9/l$	7.4 \pm 1.4	7.1 \pm 1.8	7.8 \pm 1.6	7.9 \pm 1.3	7.8 \pm 1.6	5.9 \pm 1.3**	8.2 \pm 2.1	5.3 \pm 1.4*
Neutrophil $\times 10^9/l$	1.33	1.36	1.58	1.50	1.58	1.00 *	2.10	1.13 *
Lymphocyte $\times 10^9/l$	5.77	5.40	5.78	5.93	5.78	4.13 *	5.69	3.46 *
Monocyte $\times 10^9/l$	0.27	0.33	0.39	0.50	0.39	0.73 **	0.33	0.66 *
Eosinophil $\times 10^9/l$	0.01	0.01	0.02	0.03	0.02	0.02	0.05	0.03
Basophil $\times 10^9/l$	0.01	0.01	0.03	0.02	0.03	0.02	0.03	0.02
Platelets $\times 10^9/l$	3.2 \pm 0.7	3.1 \pm 0.6	3.5 \pm 0.9	3.9 \pm 0.6	3.5 \pm 0.9	3.9 \pm 0.6	4.2 \pm 0.4	4.4 \pm 0.5

TABLE I. — Hematological findings from control (n=30) and exposed animals (n=30) to 3 μ T electromagnetic field 60 Hz according to exposure time (15, 30, 90 and 120 days). Results are expressed as means \pm standard deviations, compared to control : *p<0.05 ; ** p<0.01 (Mann Whitney U test).

No significant variation of blood parameters was evidenced between exposed and control animals at the 15th and 30th days. But, on day 90, hematocrit values, hemoglobin concentration, and red blood cell counts significantly decreased in the exposed group ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively), whereas on day 120, these parameters slightly increased (but not significantly) and were comparable to control values at the same time. Consequently, a weak macrocytosis (evidenced by a significant increase of mean corpuscular volume, $p < 0.05$) was associated to the observed anaemia, but the mean corpuscular hemoglobin concentration remained normal.

Moreover, marked decreases of total leukocyte, neutrophil and lymphocyte counts were observed at the 90th day in the exposed animals ($p < 0.01$, $p < 0.05$, $p < 0.05$, respectively), whereas in this group, monocyte counts were significantly enhanced ($p < 0.01$). Besides, all these significant alterations of white cells were still detected at the 120th day of pulsed EMF exposure. The other parameters (eosinophil, basophil and platelet counts) were not significantly different from control values at the same periods.

Discussion

In this experimental study, the effects of 60 Hz and 3 μ T electromagnetic field on some blood parameters were investigated in mice.

All parameters obtained from the control group were well within normal physiological range [9], although some blood parameters (RBC, hemoglobin concentration, WBC, neutrophil and lymphocyte counts) were gradually modified with the age of mice.

In the present study, hematocrit values and erythrocyte counts fell after 90 days of exposure, suggesting an anaemia. This anaemia was confirmed by a marked decrease of hemoglobin concentration at the same time, and was characterized by the development of macrocytosis (significant increase of mean corpuscular volume). Our results are in agreement with those previously reported by BONHOMME *et al.* [3] in mice, but they used a 5 μ T intensity. This erythrocyte abnormality would probably be due to folate or iron deficiency. In one experimental study [4], electromagnetic field has been reported to reduce concentration of iron in dairy cows. Ferrokinetic studies demonstrated that iron metabolism was affected and that erythrocyte production was significantly decreased in rabbits exposed to EMF [6]. The effects seen after 37 days of irradiation with a pulsed EMF were comparable in magnitude to those seen after 79 days exposure to a continuous-wave EMF.

On day 120, erythrocyte counts, hematocrit, hemoglobin and MCV values had increased in the exposed mice compared to the control group, suggesting compensation by the hematopoietic system, but no significant difference was obtained with the controls because of the physiological fluctuations of these parameters with the age of mice.

After 90 days of pulsed EMF exposure, severe alterations of leukocytes were observed : total leukocyte, neutrophil and lymphocyte counts were dramatically lowered whereas a

weak monocytosis was also found. Furthermore, these modifications have persisted until the 120th day. These findings were supported by BONHOMME *et al.* [3], who reported that low frequency (50 Hz and 5 μ T) electromagnetic field has decreased leukocyte and neutrophil counts. Similarly, a reduction of leukocyte number was also reported by BAYER *et al.* [1]. But, in our study, the increase of monocyte counts was also detected. However, contrary to our finding, a significant increase of neutrophil count [5] was found in rats exposed to 50 Hz, for 8 weeks. The change in neutrophil counts may be related to frequencies of EMF. The observed leucopenia (especially the neutropenia and the lymphopenia) could result from EMF effects on pluripotent marrow progenitors. It is possible that EMF acts on growth factors of hematopoietic stem cells. BONHOMME *et al.* [3] reported that low frequency EMFs caused a significant decrease of the granulocyte/ macrophage colony-forming cell (GM-CFC) concentrations. The lactoferrin (Lf), an iron binding glycoprotein, has an inhibitor role on granulocyte/ macrophage colony-forming cell production via interleukin-1 (IL-1) [15]. PENCO *et al.* [15] suggested that Lf plays a negative role in GM-CFC expression at the transcriptional level, perhaps through the mediation of IL-1 beta.

On the other hand, SELMAOUI *et al.* [16] found no significant difference of hematologic parameters between healthy and exposed men to 50 Hz magnetic fields. Similarly, MARGONATO *et al.* [12] failed to demonstrate significant changes in the hematological variables in rats exposed to EMF (50 Hz for 1240 hours). Discrepancies between findings of the various investigators may depend on a variety of characteristics of field exposure, such as frequencies, intensities, duration of exposure, field changes, and animal species.

In conclusion, this study provided evidence that chronic exposure to low-frequency EMFs (50 Hz and 3 μ T) can cause hematologic disorders in mice.

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