Exposure to 60 Hz magnetic fields does not alter clinical progression of LGL leukemia in Fischer rats

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Associations between exposure to 60-Hz magnetic fields in residential and occupational environments and the incidence of leukemia and other cancers has been suggested by the results of a number of epidemiology studies. To address these potential associations, a study has been conducted to determine if 60-Hz magnetic fields can alter the clinical progression of leukemia. In the large granular lymphocytic (LGL) leukemia model, spleen cells from aged leukemic rats were transplanted into young, male Fischer 344 rats, producing leukemia in a relatively short period. A total of 72 animals were randomly assigned to four treatment groups (18/group) as follows: (1) 10 G; (2) sham exposed (null energized field) (~20 mG); (3) ambient controls (<1 mG); and (4) positive controls (5 Gy whole body irradiation from Cobalt-60, 4 days before initiation of exposure). At the initiation of exposure or sham-exposure, all rats were injected (i.p.) with 2.2×107 fresh, viable, LGL leukemia cells. The magnetic fields were activated for 20 h per day, 7 days per week; all exposure conditions were superimposed over the natural ambient magnetic field. Eighteen rats from each treatment were bled at weeks 0, 2, 4, 5, 6, 7, 8 and 10 to monitor, in the same set of animals, the clinical progression of the LGL disease and survival of the animals. Peripheral blood hematological changes were monitored to evaluate the progression of the leukemia. In general, no significant or consistent differences were detected between the magnetic field exposed and the ambient field control groups, although some inconsistent and random differences were occasionally observed. These data indicate that the 10 G magnetic fields did not significantly alter the clinical progression of LGL leukemia in Fischer 344 rats.

Introduction

The widespread use of electric power and consequent ubiquitous exposure of human populations to power frequency electromagnetic fields (EMF*) have raised concerns that cancer risks may be associated with such exposure (1,2). Scientific evidence related to the potential effects of extremely low frequency (ELF) electromagnetic fields on the induction of cancer has been recently reviewed (3-5). Evidence to date does not support a definitive role of ELF electromagnetic fields in the clinical progression of leukemia. In the large granular lymphocytic (LGL) leukemia model, spleen cells from aged leukemic rats were transplanted into young, male Fischer 344 rats, producing leukemia in a relatively short period. A total of 72 animals were randomly assigned to four treatment groups (18/group) as follows: (1) 10 G; (2) sham exposed (null energized field) (~20 mG); (3) ambient controls (<1 mG); and (4) positive controls (5 Gy whole body irradiation from Cobalt-60, 4 days before initiation of exposure). At the initiation of exposure or sham-exposure, all rats were injected (i.p.) with 2.2×107 fresh, viable, LGL leukemia cells. The magnetic fields were activated for 20 h per day, 7 days per week; all exposure conditions were superimposed over the natural ambient magnetic field. Eighteen rats from each treatment were bled at weeks 0, 2, 4, 5, 6, 7, 8 and 10 to monitor, in the same set of animals, the clinical progression of the LGL disease and survival of the animals. Peripheral blood hematological changes were monitored to evaluate the progression of the leukemia. In general, no significant or consistent differences were detected between the magnetic field exposed and the ambient field control groups, although some inconsistent and random differences were occasionally observed. These data indicate that the 10 G magnetic fields did not significantly alter the clinical progression of LGL leukemia in Fischer 344 rats.

*Abbreviations: EMF, electromagnetic fields; ELF, extremely low frequency; RBC, red blood cell; WBC, nucleated blood cell; VPRC, volume of packed red cell; nRBC, nucleated RBC.

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chemicals testing positive for cancer in lifetime animal studies (e.g. trichlorophenol, ethylene glycol) and those testing positive using a spleen cell transplant model have been reported (17,18).

Regardless of the direct relevancy of LGL leukemia to humans, this model should provide important information on late-stage events in carcinogenesis specific to leukemia. Thus, the purpose of this study is to determine if 60-Hz magnetic fields can alter the progression of leukemia in young Fischer-344 male rat recipients of spleen cells from donor rats showing signs of large granular lymphocytic (LGL) leukemia.

Transplantable LGL model
Male Fischer 344 rats have a high spontaneous incidence of LGL leukemia in old age. When leukemic spleen cells from these rats are transplanted into young rats, the leukemic disease develops in 7 to 12 weeks, depending on the number of cells injected. Spleen cells were harvested from spontaneous donors and a pool of cells was established for use in this model. We have characterized the model for disease progression with time, determined the relationship between quantity of cells injected and disease progression, and established an effective positive control (19). The time course of the disease can be altered or manipulated by judicious selection of the number of cells used in the transplant and by whole body irradiation with Cobalt-60 gamma radiation.

Materials and methods

Animals
Male Fischer 344 rats ~5 weeks of age were obtained from Charles River Laboratory (Raleigh, NC) and fed a standard diet (NIH-07 Open Formula pellets) and water ad libitum. A pre-exposure viral antibody health screen, including a gross necropsy and selected histopathology and serological testing for the presence of potentially pathogenic organisms, revealed no adverse health problems in ten randomly selected male rats.

Animals were individually housed in polycarbonate cages on the magnetic field exposure systems, and were allowed to acclimatize to their environment for 1 week before the start of exposure. Room temperatures and relative humidity were taken every 5 min by computer and the average values summarized daily. Daily values were maintained within the desired limits (temperatures 73 ± 3°F and humidity 55 ± 15%) throughout the study. Animal rooms were illuminated by fluorescent lights, controlled by automatic timers, on a 12-hour light/12-hour dark cycle. Lights were set to come on at 06:00 and off at 18:00 h daily.

Experimental procedure
A total of 72 animals were randomly assigned to four treatment groups (18/group) as follows: (1) 10 G (1.0 mT) linearly-polarized 60 Hz magnetic field; (2) sham exposed (null energized module) with a residual ~20 mg (2 μT) field; (3) ambient controls [<1 mG (0.1 μT)]; and (4) positive controls (5 Gy whole body irradiation from Cobalt-60, 4 days before initiation of exposure).

The animals were individually identified by tail tattoo and assigned to treatment groups by using body weight as a blocking variable (average body weight was 126.0 g). Rats in this and a companion study were housed on both sides of the room. The ambient systems are in a separate room in the same facility.

Exposure system
Exposures were performed in a small-animal magnetic field exposure system that provided 60-Hz sinusoidal magnetic fields, linearly polarized in the horizontal direction. This system has been characterized (i.e. documentation of frequency and waveform intensity, as well as quantification of possible confounding factors produced by the system such as: field uniformity, heating, harmonic distortion and noise, audible hum, electric fields, and stray magnetic fields) (20). The exposure system consists of four units, each with six 1.05 m side. Rats in this and a companion study were housed on both sides of the exposure/sham exposure systems.

The coil windings are double-wound (essentially as two separate coils within each coil structure) throughout the study for longitudinal evaluation of the clinical progression of the LGL disease. Exposure system
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### Table I. 60 Hz Magnetic field levels for three treatment groups*

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>N</th>
<th>Mean (mG)</th>
<th>Standard deviation (mG)</th>
<th>Range (mG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed</td>
<td>72</td>
<td>10.2 G</td>
<td>0.37 G</td>
<td>9.5-10.8 G</td>
</tr>
<tr>
<td>Sham (null field)</td>
<td>72</td>
<td>17.8 mG</td>
<td>14.9 mG</td>
<td>4.6-61.6 mG</td>
</tr>
<tr>
<td>Ambient control</td>
<td>60</td>
<td>0.76 mG</td>
<td>0.10 mG</td>
<td>0.60-1.0 mG</td>
</tr>
<tr>
<td>Positive control</td>
<td>60</td>
<td>0.96 mG</td>
<td>0.21 mG</td>
<td>0.60-1.3 mG</td>
</tr>
</tbody>
</table>

*The exposure field was essentially unidirectional (horizontal). The Sham and Ambient Control group fields, however, were the three axis resultant fields.

Number of individual positions measured in the exposure systems.
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- Fig. 1. Average body weights (mean ± SE) of rats injected with 2.2×10⁷ LGL cells. The growth curve of the positive control group was significantly different (P < 0.05) from the other treatment groups.

- Fig. 2. Percent of animals with palpable spleens during the study. Splenomegaly was detected in the positive control animal about 1 week earlier than in the control or magnetic field exposed animals.

subsequently at weeks 2, 4, 5, 6, 7, 8 and 10. An Ortho ELT-8/ds multiparameter blood analyzer was used to perform the red blood cell (RBC) and total nucleated blood cell (WBC) counts, hemoglobin and volume of packed red cell (VPRC) determinations, and red cell indices. Leukocyte differential counts, including LGL cells, were made from slides stained with Wright-Giemsa.

Statistical methods

The hypothesis to be tested in this study was that exposure of rats to 60 Hz magnetic fields enhances the clinical progression of leukemia after inoculation of the test animals with large granular lymphocytic (LGL) leukemia cells. Statistical procedures from the Statistical Analysis Systems Institute (21) software library were used to perform most of the data analysis. Analysis of variance was used to analyze measurements made on continuous variables. Appropriate transformations were utilized where the variable measured was not normally distributed. Tukey's post hoc test was used to delineate intergroup differences. Repeated measures analysis of variance was used to analyze variables repeated over time on the same animal. Analysis of measurements on binary response variables were evaluated by chi-square tests. Pairwise comparisons in the case of significant findings were made by Fisher's exact test. The product-limit method was used to analyze the survival data (22) and survival curve comparisons were made using the generalized Wilcoxon test.
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Fig. 3. Survival curves of rats injected with $2.2 \times 10^7$ LGL cells. A significant ($P < 0.05$) life-shortening was observed for the positive control animals compared with the control or magnetic field exposed animals.

Results

Animals receiving the spleen cell transplants were observed for characteristic disease signs such as behavioral, respiration, skin and hair coat, ocular changes, evidence of diarrhea, weight loss, palpable (enlarged) spleens, and morbidity throughout the study. In general, the time course for disease onset was about 7 to 12 weeks. Typical signs of the disease included splenomegaly, thin condition, and rough hair coats. Animals near death were usually listless and hunched-up with evidence of urine stain on the abdominal surface. Jaundice was occasionally observed.

Growth and survival

The growth curves of this repeated-bleeding study are presented in Figure 1. Rats of all treatment groups, except positive controls, grew at an almost constant rate until about 56 days of treatment, after which growth generally ceased and a weight loss was observed for all three treatment groups, with no significant difference among groups. The growth curve of the positive control group was significantly depressed throughout the study ($P < 0.05$) compared with the other three groups. Growth retardation began sooner and was more severe for the positive control group than the treatment groups after the onset of the leukemia.

The progression of splenomegaly as determined by palpable spleens is shown in Figure 2. Differences were not observed between controls and the 10 G or sham exposed (null field) groups, but enlarged spleens were detected in a majority of the positive control group about 7 days earlier than in the other treatment groups. Over 90% (67 of 72) of the rats were palpated positive by 63 days of exposure. All of the animals in the study were palpated positive by the end of the study, although one animal in the ambient control group was not positive until the final week of the study. These results were confirmed with spleen weights at necropsy. Spleen weights at death ranged from about 4–25 g in the three magnetic field treatment groups, whereas spleen weights of the positive control group were much smaller (~3–13 g), even though the expression of the disease as indicated by other biological markers (including early palpable spleens) were more severe in the positive control than in the ambient control animals.

A comparison of the survival curves for the four groups showed that only the positive control group was significantly different ($P < 0.05$; Figure 3). The mean survival estimates were about 115 days for the treatment groups (10 G, sham field, and ambient controls) and 99 days for the positive controls. However, these estimates are somewhat biased because only 1 to 7 rats per group were still alive at the termination of the study (day 126). The three earliest deaths (one in the 10 G group and two in the positive control group) resulted from hemorrhaging due to fractured spleens. One animal (10 G group) was killed in a moribund paralyzed condition at day 93. Correction for these deaths were not made in the data presented in Figure 3, as inclusion or exclusion of these data do not alter the interpretation of the survival results.

Hematology

The erythrocyte parameters (hemoglobin concentration, RBCs and VPRC) generally demonstrated a pronounced decline between 7 and 10 weeks after cell injection. These results confirm the clinical manifestation of anemia in these animals with the advance of the leukemia.

Significant differences were not generally observed among the exposed, sham exposed, or control groups (especially during onset and progression phases of the disease), but RBC and hemoglobin concentration were significantly reduced ($P < 0.05$) for the positive control group (Figure 4). A similar pattern was observed for VPRC (data not shown). Analysis of variance indicated occasional and isolated minor differences among treatment groups, primarily before week 7 where only small changes had occurred due to the progression of leukemia. The occasional small and inconsistent differences may partly
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Hemoglobin and RBC (Figure 4) as well as VPRC of the positive control group were significantly depressed \((P < 0.05)\) after irradiation. Hemoglobin concentration and VPRC recovered by 28 days (prior to the onset of the leukemia), but the number of RBCs were significantly \((P < 0.05)\) depressed throughout the study.

A significant increase \((P < 0.05)\) in the percent nucleated RBC (nRBC) of all nucleated cells began 8 weeks after injection of all treatment groups (Figure 4). Treatment-related differences were not observed, but the percent of nRBC in peripheral blood for the positive control group increased significantly \((P < 0.05)\) at week 8 and then decreased compared with controls.

The greatest impact of the disease on the nucleated cells was due to increases in nRBC and LGL cells. Nucleated blood cell counts were adjusted for the number of nRBC then expressed as a corrected WBC. WBC and LGL cells of the treated groups remained in the normal range through 7 weeks after which there was a sharp rise at 8 weeks post-injection (Figure 5). An increase in percent LGL was detected 1 week earlier. Absolute lymphocyte values tended to rise slightly at 10 weeks (Figure 5), but the percent lymphocytes declined rather sharply after 6 weeks (data not shown).

WBC and LGL cells were significantly greater \((P < 0.05)\) in positive control rats compared with the ambient controls at 8 weeks (Figure 5). Lymphocytes were depressed following irradiation but, after rebounding, became elevated by 8 weeks compared with the controls. No consistent changes occurred in other differential blood parameters that would suggest an effect of exposure to the magnetic field.

Discussion

The progression of the LGL leukemia in these rats developed as expected and is generally in agreement with the description by Stromberg (10). The number of spleen cells for transplantation was selected to maximize the number of successful transplants over a short time span. Using this design, the onset of the disease was recorded in most animals within a period of about 1 week. The leukemic rats had marked hemolytic anemia, associated with severe reticulocytosis and slight increases in MCV and MCH. Leukocytosis, associated with disease development, was...
mostly the result of the proliferation of LGL cells in the peripheral blood. Thrombocytopenia generally occurred in parallel with the anemia, but did not develop with a sudden onset. These hematological changes were accompanied by the development of splenomegaly and a loss in body weight.

Results of the positive control group demonstrate significant and consistent differences between positive controls and the ambient control group for many of the endpoints evaluated. This suggests that the model was sensitive to the effects of an insult (ionizing radiation), consistent with our previously reported results (19), and adds confidence to the negative results found with the 10 G magnetic field exposure animals.

Thomson et al. (9) also reported that exposure to magnetic fields did not effect the incidence or progression of leukemia in an animal transplant model. In this case, mice were implanted with P388 leukemia cells and exposed to the fields for 6 h/day, 5 days/week. However, in this particular leukemia model, the animals only survived for about 2 weeks after the cells were transplanted, allowing a very short period for demonstrating differences between the exposed and control animals. The study did not include positive controls for demonstrating whether or not the model could be manipulated.

This study of the progression of leukemia in rats transplanted with leukemic spleen cells and exposed to 10 G or sham (nulled) magnetic fields did not demonstrate significant and consistent effects on the progression of LGL leukemia in the Fischer rat. There were no differences in body weight or survival between the exposed and the unexposed rats at any time in the study to suggest a significant effect of exposure on the progression of the transplanted disease. Although minor differences were occasionally observed in the hematological parameters between exposed and unexposed groups, the consistency among endpoints measured and the extremely small magnitude of the differences observed suggest that they were random or chance associations. These data indicate that the 10 G magnetic fields did not significantly alter the clinical progression of LGL leukemia.

Acknowledgements

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