Electromagnetic fields enhance chemically-induced hyperploidy in mammalian oocytes

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Epidemiological studies suggest that exposure to electromagnetic fields (EMFs) in the environment may be associated with mutagenic changes, but the relation between EMF exposure and aneuploidy has not previously been studied. Environmental EMFs apparently lack the energy necessary to function as aneugens, but the possibility exists that EMFs could influence the incidence of aneuploidy synergistically because EMFs can activate the neuroendocrine system, and ovulation and oocyte meiotic maturation are under neurohormonal control. This hypothesis was tested by examining the effect of EMF exposure on the occurrence of hyperploidy in mouse oocytes induced by vinblastine sulphate (VBS), which was employed as a surrogate for aneugens in the environment. The incidence of hyperploidy in metaphase II oocytes of individual mice following superovulation was determined, and statistical methods were developed to assess whether EMF exposure during oogenesis in the presence of VBS altered the rate of hyperploidy. A significant effect of EMF exposure on VBS-induced hyperploidy was found (P < 0.05). The data suggested that the EMF primarily affected the mice that exhibited a high incidence of VBS-induced hyperploidy. Exposure had no effect on the number of oocytes ovulated nor on the occurrence of hypoploidy. The results support the hypothesis that EMF exposure can promote the occurrence of aneuploidy caused by an aneugen via a mechanism involving the neuroendocrine system.

Introduction

The genetic consequences of exposure to electromagnetic fields (EMFs) in the environment are generally unknown, and there is a consequent need for additional information (Murphy et al., 1993). Some epidemiological studies suggested an association between EMFs and cancer, particularly cancer of the blood-forming and nervous systems (Marino, 1993). The possible teratogenic effects of EMFs have not been examined directly in epidemiological studies, but an association between the occurrence of spontaneous abortion and exposure to EMFs has been reported (Wertheimer and Leeper, 1986). Animal studies showed that EMFs can be teratogenic under certain circumstances (Juutilainen et al., 1986; Berman et al., 1990). There is apparently no epidemiological evidence concerning the possibility of a link between EMFs and aneuploidy, except for reports of a possible relationship between Down's syndrome and paternal radar exposure (Cohen and Lilienfeld, 1970; Cohen, 1976).

Most attempts to find evidence of direct genetic effects that could plausibly serve as a basis for the epidemiological associations have generally been unsuccessful (Reese et al., 1988; Saunders et al., 1988; Frazier et al., 1990; Garcia-Sagredo et al., 1990; Dertinger et al., 1993; McCann et al., 1993; Scarfi et al., 1993). The failure to find direct evidence of EMF-induced mutagenic effects is consistent with biophysical modelling, which does not predict that environmental-strength EMFs have sufficient energy to break chemical bonds (Adair, 1991). It seems unlikely, therefore, that EMFs are mutagens in the sense of initiating mutagenic transformations via a direct interaction with nucleic acids as can occur in the case of ionizing radiation or some chemical agents.

Another approach involves consideration of possible modulatory effects of EMFs on a genetic effect caused by another factor. As examples, EMFs had no effect on UV light-induced mutations in Saccharomyces cerevisiae (Ager and Radul, 1992), but animals treated with 7.12-dimethylbenz[a]anthracene (DMBA) and exposed to an EMF exhibited increased tumour incidence, compared with DMBA-treated non-exposed controls (Baum et al., 1995; Mevissen et al., 1995).

Since EMFs are probably not mutagenic but can affect the development of cancer as shown by the DMBA studies, it seemed reasonable to expect that any link with aneuploidy was similarly likely to involve modulation or promotion rather than initiation. We therefore considered the question whether EMFs were capable of modulating the level of aneuploidy induced in animals by a known aneugen, using an established mammalian female germ cell model and vinblastine sulphate (VBS) as the aneugen (Mailhes et al., 1988). It was found that EMFs significantly enhanced the incidence of hyperploidy induced by VBS in mouse oocytes.

Materials and methods

Animals

Since we postulated that the effects of EMFs on VBS-induced aneuploidy would occur as a consequence of changes in the neuroendocrine system, the animal and not the oocyte was viewed as the basis for assessment of an effect, and the level of aneuploidy exhibited by individual animals was studied.

Virgin female ICR mice (Harlan Sprague-Dawley Inc) 8–12 weeks of age (25–34 g) were maintained under a 12 h light–12 h dark period (light commencing at 06:00) at 21–23°C, and 50 ± 5% relative humidity. The animals were housed in a totally non-metallic environment and were fed and watered ad libitum. Maturation of ovarian follicles was augmented by an i.p. injection of 7.5 U pregnant mare's serum (PMS, Foligini; Intervet), and 48 h later ovulation was induced by an i.p. injection of 5 U human chorionic gonadotrophin (HCG; Ayerst Inc).

VBS (CAS #143-67-9, Cetus) was chosen as the aneugen because our previous work established that it reproducibly increased the incidence of aneuploidy in mouse oocytes (Mailhes and Marchetti, 1994). Since the goal was to evaluate the effect of EMFs on the aneuploidy induced by VBS, all mice entered into the study received an i.p. injection of 0.2 mg/kg VBS, which was given when the HCG was administered. Half the mice were then exposed to the EMF, and half were sham-exposed.

EMF exposure

Power-frequency fields are commonly present in the environment, but field strengths >5 G are rare; consequently, a magnetic field of 5 G, 60 Hz was chosen for study. The field was generated using a pair of Helmholtz coils 1.3 m in diameter (exposure unit), and was homogeneous to within 5%
change in temperature (<0.3°F) at the location of the mice. Coil current was
unit, similar in all respects to the exposure unit except for the absence of
(Krohn-Hite Model 7500) or an adjustable autotransformer. A sham-exposure
obtained from either a signal generator (Wavetek Model 182A) and amplifier
resonance to minimize power dissipation (75 W), and produced no detectable
throughout the space occupied by the mice. The coils were operated in series
Fig. 2. Effect of EMF on frequency distribution of hyperploidy in mice. (A) 0–10% hyperploid (16 mice); (B) 10.1–20% hyperploid (12 mice); (C) >20% hyperploid (seven mice). The data from two mice (five cells or fewer could be analysed) in the EMF + VBS group and three mice (five cells or fewer could be analysed in one mouse and failure to ovulate in two mice) in the VBS group were excluded.

Table II. Effect of EMF exposure on vinblastine-induced aneuploidy. Mean percentage ± SD of oocytes from each animal with hyperploid or hypoploid MII oocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean</th>
<th>L_2</th>
<th>SD</th>
<th>L_1</th>
<th>L</th>
<th>P(L)</th>
</tr>
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<tbody>
<tr>
<td>Hyperploidy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMF + VBS</td>
<td>18</td>
<td>15.0</td>
<td>1.22</td>
<td>12.3</td>
<td>8.85</td>
<td>10.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VBS</td>
<td>17</td>
<td>11.4</td>
<td>6.9</td>
<td>5.9</td>
<td>2.85</td>
<td>3.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EMF + VBS</td>
<td>18</td>
<td>19.5</td>
<td>14.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoploidy</td>
<td></td>
<td></td>
<td>0.62</td>
<td>11.8</td>
<td>0.68</td>
<td>1.30</td>
<td>NS</td>
</tr>
<tr>
<td>VBS</td>
<td>17</td>
<td>22.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMF + VBS</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

NS, not significant.

(total time, ~3 h from animal death). The chromosomes were C-banded to facilitate discrimination between dyads and single chromatids (Salamanca and Armandes, 1974). To evaluate whether the preparative procedures differentially affected the cells available for analysis, we recorded: (i) the number of oocytes ovulated; (ii) the number of oocytes successfully mounted on the microscope slide; and (iii) the number of recovered cells that could be analysed for numerical and structural aberrations.

Oocytes were excluded from analysis if the C-banding was insufficient to permit visualization of the centromeres or if the chromosomes of individual cells were either excessively scattered or clumped. The number of polyplody and metaphase I (MI) oocytes were recorded, but were excluded from the calculation of aneuploidy.

Cytogenetic analysis

A total of 40 mice were entered into the study, half in each treatment group. For each animal, each metaphase II (MII) oocyte was examined at ×1250 and the numbers of haploid (n = 20), hyperploid (n = 10–19.5), hyperploid (n = 20.5–29.5), polyploid (n = 30–40) and MI oocytes were recorded, along with their rectilinear coordinates on the slide. The frequencies (expressed as percentages) of haploid, hypoploid and hyperploid MII oocytes were calculated for each mouse as the ratio of the number of each cell type to the total number of MI oocytes analysed. Animals with too few MI oocytes (five or less) were excluded from the assessment of the effect of the field on ploidy frequency; this condition resulted in the exclusion of two VBS + EMF mice and one VBS mouse. In addition, no data were obtained from two VBS mice which failed to ovulate. The frequency of hyperploid MII oocytes was used to estimate aneuploidy because an unknown proportion of hypoploid results from chromosome loss during slide preparation (Mailhes and Marchetti, 1994; Mailhes, 1995).

Statistical analysis

Previous reports suggested that, characteristically, the effects caused by low-strength EMFs were likely to be mediated by the central nervous system and to occur in only some animals in the exposed population (Marino, 1988, 1995). A statistical hypothesis was therefore developed to directly test this theory. Let μ_1 and σ_1 denote the mean and variance in the ploidy frequency of the VBS + EMF mice respectively; μ_2 and σ_2 are the corresponding quantities in the VBS group. The statistical hypothesis was that the two distributions were identical, that is, μ_1 = μ_2 and σ_1 = σ_2. The hypothesis σ_1 = σ_2 is commonly tested using the statistic F = s_1^2/s_2^2 with N_1 – 1 and N_2 – 1 degrees of freedom, and μ_1 = μ_2 is commonly tested using the t statistic with N_1 + N_2 – 2 degrees of freedom. The likelihood approach allows the statistics to be combined into a single statistic L = L_1 + L_2, where L_1 and L_2 are the log-likelihood ratio statistics for the variance and mean respectively (Anderson, 1984).

L_1 = N_1 \ln \left( \frac{N_1}{N_1 + N_2} \right) \left( 1 + \frac{(N_1 - N_2 \bar{s}_1^2)}{(N_1 - 1)\bar{s}_1^2} \right) + N_2 \ln \left( \frac{N_2}{N_1 + N_2} \right) \left( 1 + \frac{(N_2 - N_1 \bar{s}_2^2)}{(N_2 - 1)\bar{s}_2^2} \right)

L_2 = (N_1 + N_2) \ln \left( 1 + \frac{1}{N_1 + N_2 - 2} \right)^2

The distribution of L is approximately χ^2 with 2 degrees of freedom. Thus (μ_1,σ_1) = (μ_2,σ_2) can be rejected if L > χ^2, where α < 0.05. The Kolmogorov–Smirnov test was used to evaluate whether particular data deviated from a normal distribution. The planned analysis of the effect of EMFs on VBS-induced aneuploidy was restricted to hyperploid oocytes because hyperploidy is less sensitive than hypoploidy to the occurrence of artefacts during slide preparation. All other pair-wise comparisons were performed using the Mann–Whitney U test, with P < 0.05 as the significance level.

Results

The cytogenetic data are presented for each mouse separately in Table I. The EMF had no effect on the mean number of oocytes ovulated (Figure 1A). Some oocytes were lost during processing and transfer to microscope slides, but the amount of loss was unaffected by the field (Figure 1B). Although some recovered MII cells could not be analysed for aneuploidy because of poor staining or excessive chromosome clumping or scattering, the number of cells which could be analysed did not differ between treatments (Figure 1C).

Cells from 18 VBS + EMF mice and 17 VBS mice were analysed for numerical and structural chromosome aberrations, and the frequency of hyperploidy in each animal was determined. A significant effect of EMF exposure on hyperploidy was found as indicated by the value of the L statistic (Table II). Most of the effect could be attributed to a difference in sample variance, as reflected in the value of L_1. This impact on
levels were adversely affected (because the number of such animals was greater when the EMF was applied). Thus, the results reported here fit the emerging pattern of EMF-induced bioeffects because they indicate: (i) only some animals were affected; (ii) manifestation of the effect was primarily in sample variance; (iii) the effect may have involved the central nervous system.

The effect of EMFs alone on hyperploidy in the absence of VBS was not considered because the goal of the study was to determine whether EMFs could modulate hyperploidy caused by an aneugen. VBS was used as a surrogate for aneugenics in the environment such as ionizing radiation or chemical agents. Nevertheless, the result reported here seems to justify an experimental verification of the physical theory holding that EMFs of the type employed here cannot induce aneuploidy (Adair, 1991).

There was no significant effect of EMFs on hypoploidy (P > 0.05). This may have resulted from preferential inclusion of chromosomes in polar bodies, but a more likely explanation is that the randomizing effects of technical artefacts introduced variability into the hypoploidy data that prevented attainment of statistical significance. The data in Table I showing greater variance in hypoploidy in comparison with hyperploidy supports this inference.

Polyplody with a block in cytokinesis occurs at a frequency of 0.4–1.9% in oocytes exposed to VBS (Mailhes et al., 1993; Mailhes et al., 1995). This suggests that the difference in hypoploidy observed in this study (EMF + VBS 5/383, VBS 0/311, Table I, P = 0.07, Fisher’s Exact test) was due to chance.

We did not address the issue of mechanism, but there are various possibilities. During oocyte meiotic maturation, the oocyte transitions from the dictyate stage to MII. Perturbations during maturation may predispose oocytes to chromosome missegregation by disrupting the orderly sequence of interrelated processes essential for normal nuclear and cytoplasmic maturation. Any agent that affects the neuroendocrine system and acts precisely at the time that the oocyte is undergoing meiosis, could affect the incidence of aneuploidy (Mailhes and Marchetti, 1994; Mailhes, 1995). Such perturbations during oocyte maturation can result from damage to organelles responsible for cell division, hormonal imbalance, physiologic ageing of the oocyte-follicle complex, interactions between endogenous and exogenous chemicals with those involved in oocyte maturation, and other yet unidentified processes. Finally, any condition that alters gamete gene expression, protein synthesis and phosphorylation states, or calcium homeostasis, has the potential for inducing aneuploidy (Racowsky, 1993; Mailhes et al., 1995).

Discussion
An effect of EMF exposure on VBS-induced hyperploidy was found as determined using the L statistic (Table II). Most of the effect was associated with the contribution to L from the variance, suggesting that EMF exposure affected only some of the exposed animals. This view was supported by an analysis of the levels of VBS-induced hyperploidy in individual animals. The EMF had no effect on the mice that displayed <10% aneuploid oocytes, as evidenced by the occurrence of equal numbers of mice in the VBS + EMF and VBS groups (Figure 2A). In contrast, there were six mice in the VBS + EMF group that exhibited hyperploidy frequencies >20%, compared with only one mouse in the VBS group (Figure 2C). Although the exact numerical results depend on how the groups are defined, all reasonable groupings tend to show that the mice with relatively low VBS-induced hyperploidy levels were unaffected by the EMF, while those exhibiting higher levels were adversely affected (because the number of such mice was greater when the EMF was applied).

No effect of EMF exposure on hypoploidy was seen (Table II). Polyplody was only found in mouse No. 2 (1/7) and mouse No. 5 (4/10) of the EMF + VBS groups. Structural aberrations were not found.

Fig. 3. (a) Mouse hyperplloid metaphase II oocyte, n = 21. (b) Mouse hyperplloid metaphase II oocyte, n = 22.
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