Electromagnetic fields enhance chemically-induced hyperploidy in mammalian oocytes

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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, Folligon; 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%...
throughout the space occupied by the mice. The coils were operated in series
to minimize power dissipation (75 W), and produced no detectable
cell current was
was obtained from either a signal generator (Wavetek Model 182A) and amplifier
(Krohn-Hite Model 7500) or an adjustable autotransformer. A sham-exposure
unit, similar in all respects to the exposure unit except for the absence of
cells, was used to house the control animals. The exposure and sham-exposure
obtained from either a signal generator (Wavetek Model 182A) and amplifier
throughout the space occupied by the mice. The coils were operated in series
and sham-exposure units were housed in the same room throughout the study; the fringing field
at the location of the sham-exposure unit was 40 ± 20 mG. The mice were
exposed or sham-exposed to the EMF for 17 h following the HCG/VBS
injections, and then killed. A period of 17 h was chosen because it is
suitably long to allow ovulation, but without significant oocyte degeneration
(Polanski, 1986; Tiveron et al., 1992).

Oocyte harvest and processing
Ovulated oocytes were collected from the oviducts and their chromosomes
analysed as described previously (Mailhes and Yuan, 1987). Briefly, for each
mouse, the ovulated oocytes were counted prior to fixation and slide preparation

Table I. Effect of EMF exposure on vinblastine-induced aneuploidy in mice. Difference between recovered and analysable indicates cells that were
evertheless clumped or scattered or did not stain properly. The parentheses in the analysable column indicate non-analysable cells that were either polyploid
or in MI. The polyploid oocytes (EMF + VBS Numbers 2 and 5) did not have a polar body

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>VBS group</th>
<th>EMF + VBS group</th>
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<tbody>
<tr>
<td></td>
<td>Ovulated</td>
<td>Recovered</td>
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<tr>
<td>1</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>18</td>
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<td>3</td>
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</table>

Fig. 1. Effect of EMFs on recovery of oocytes from mice treated with vinblastine sulphate (mean ± SE). (A–C), number of oocytes ovulated, recovered, and
that can be analysed respectively. n = 20 and 18 for the EMF + VBS and VBS group respectively.
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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).

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>
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<tr>
<td>VBS</td>
<td>17</td>
<td>11.4</td>
<td>5.9</td>
<td>10.8</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>EMF + VBS</td>
<td>18</td>
<td>19.5</td>
<td>14.4</td>
<td>10.8</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
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<tr>
<td>Hypoploidy</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VBS</td>
<td>17</td>
<td>22.9</td>
<td>11.8</td>
<td>1.30</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant.

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.

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 X1250 and the numbers of haploid (n = 20), hypoploid (n = 10-19.5), hyperploid (n = 20.5-29.5), polyplol (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 MI 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 MI oocytes was used to estimate aneuploidy because an unknown proportion of hypoploid results to occur in only some animals in the exposed population (Marino, 1988, 1995). 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 \( \mu_1 \) and \( \sigma_1 \) denote the mean and variance in the ploidy frequency of the VBS + EMF mice respectively; \( \mu_2 \) and \( \sigma_2 \) are the corresponding quantities in the VBS group. The statistical hypothesis was that the two distributions were identical, that is, \( \mu_1 = \mu_2 \) and \( \sigma_1 = \sigma_2 \). The hypothesis \( \sigma_1 = \sigma_2 \) is commonly tested using the statistic \( F = \frac{s_1^2/s_2^2}{N_2 - 1} \) with \( N_2 - 1 \) and \( N_2 - 1 \) degrees of freedom, and \( \mu_1 = \mu_2 \) 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).

\[
L_1 = N_1 \ln \left( \frac{N_1}{N_1 + N_2} \left( 1 + \frac{(N_2 - 1)s^2}{(N_1 - 1)s^2} \right) \right) + N_2 \ln \left( \frac{N_2}{N_1 + N_2} \left( 1 + \frac{(N_1 - 1)s^2}{(N_2 - 1)s^2} \right) \right)
\]

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

The distribution of \( L \) is approximately \( \chi^2 \) with 2 degrees of freedom. Thus \( (\mu_1, \sigma_1^2) = (\mu_2, \sigma_2^2) \) can be rejected if \( L > \chi^2_{2,\alpha} \) where \( \alpha < 0.05 \). 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.

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 \( \mu_1 \) and \( \sigma_1 \) denote the mean and variance in the ploidy frequency of the VBS + EMF mice respectively; \( \mu_2 \) and \( \sigma_2 \) are the corresponding quantities in the VBS group. The statistical hypothesis was that the two distributions were identical, that is, \( \mu_1 = \mu_2 \) and \( \sigma_1 = \sigma_2 \). The hypothesis \( \sigma_1 = \sigma_2 \) is commonly tested using the statistic \( F = \frac{s_1^2/s_2^2}{N_2 - 1} \) with \( N_2 - 1 \) and \( N_2 - 1 \) degrees of freedom, and \( \mu_1 = \mu_2 \) 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).

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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 aneugens 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.

Polyploidy 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 polyploidy 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).

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