Effect of 26 week magnetic field exposures in a DMBA initiation–promotion mammary gland model in Sprague–Dawley rats

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Several studies have suggested that exposure to 50 Hz magnetic fields promote chemically induced breast cancer in rats. Groups of 100 female Sprague–Dawley rats were initiated with a single 10 mg gavage dose of 7,12-dimethylbenz[a]anthracene (DMBA) at 50 days of age followed by exposure to ambient fields (sham exposed), 50 Hz magnetic fields at either 1 or 5 Gauss (G) field intensity or 60 Hz fields at 1 G for 18.5 h/day, 7 days/week for 26 weeks. A vehicle control group without DMBA was included. Rats were palpated weekly for the presence of tumors. There was no effect of magnetic field exposure on body weight gains or the time of appearance of mammary tumors. At the end of 26 weeks, the animals were killed and the mammary tumors counted and measured. Mammary gland masses found grossly were examined histologically. The mammary gland carcinoma incidence was 96, 90, 95 and 85% (P < 0.05, decrease) for the DMBA controls, 1 G 50 Hz, 5 G 50 Hz and 1 G 60 Hz groups, respectively. The number of tumors varied from 276 to 319, with the lowest number in the 1 G 60 Hz exposure group. Measurement of the tumors revealed no difference in tumor size between groups. In this breast cancer initiation–promotion study in female Sprague–Dawley rats, there was no evidence that 50 or 60 Hz magnetic fields promoted breast cancer under the conditions of this assay. This study does not support the hypothesis that magnetic field exposure can promote breast cancer in this rat model.

Introduction

Electric and magnetic fields associated with the production, transmission, and use of electricity are ubiquitous in industrialized society. These electric and magnetic fields are predominantly of low frequency (60 Hz in the US, 50 Hz in Europe and Japan) and generally of low intensity. Electric fields exist when there is electric potential in a line, while magnetic fields exist only when there is current flow. Since both electric and magnetic fields often occur together and are interactive, these fields have often been referred to as electric and magnetic fields or EMFs. Electric fields are easily shielded by trees, walls and other objects while magnetic fields usually penetrate non-ferrous material. Thus, most residential exposure is to magnetic fields. More recent research has focused on potential adverse biological effects of exposure to magnetic fields. The residential exposures in most homes are to magnetic field intensities of <2 milligauss (mG) which is equivalent to 0.2 microtesla (µT) although some areas in homes may exceed this field intensity. In certain industrial settings, mean workplace magnetic field exposure may exceed 10 mG (2).

Concern that these low frequency fields could alter the rates of breast cancer in humans was raised by the report of cases of breast cancer in male electricians, especially since breast cancer in men is such a rare disease (3,4) and more recently by a report of a modest increase in breast cancer in women with magnetic field exposure (5). While male breast cancer is rare, any effect of EMF on the incidence of breast cancer in women has potential major health implications. Furthermore, the suggestion that magnetic field exposure may depress nocturnal melatonin levels (6) provided a plausible mechanism for alterations in breast cancer rates.

Several studies have failed to find an effect on long term magnetic field exposure on mammary gland tumor rates in rodents (7–9). Other reports suggested that the 50 Hz magnetic fields may promote 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary gland cancer in female rats (10–16). In an attempt to confirm whether magnetic fields could promote breast cancer in rats, we used a DMBA initiation–promotion model in Sprague–Dawley rats with similar magnetic field exposure conditions. We also used the American power line frequency (60 Hz) in addition to the 50 Hz European frequency.

Since most initiation–promotion studies use a single dose of DMBA (17–19), we evaluated a single dose of 10 mg of DMBA, followed by 26 weeks of magnetic field promotion to test potential promoting effects of these fields on breast cancer. The results of the 26 week studies are reported here.

Materials and Methods

Animals

Female Sprague–Dawley rats were obtained from Charles River Laboratory (Raleigh, NC). On receipt, the animals were 37 days old. The animals were quarantined for 13 days and were 50 days of age on the first day of the study. Before the initiation of the study, 10 rats were randomly selected for parasite evaluation and gross observation for evidence of disease. The study animals were randomized by weight into treatment groups using a computer generated randomization scheme. At the end of the study, serological analysis were performed on five rats from each of the two exposure rooms. Serological evaluation for Sendai virus, pneumonia virus of mice, rat coronavirus/shalodacroadenitis virus and Kilham rat virus/H-1 virus failed to reveal any abnormalities either prior to, or at the end of, the study.

Animal room conditions

Rats were housed 5 per polycarbonate cage (23×15×8 ins) on hardwood bedding (P.J.Murphy Forest Products, Montville, NJ); cages were changed daily.

Abbreviations: DMBA, 7,12-dimethylbenz[a]anthracene; EMF(s), electric and magnetic field(s); G, Gauss; H&E, hematoxylin and eosin; mG, milligauss; µT, microtesla.

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twice weekly, and water and NIH-07 open formula pelleted diet (Ziegler Brothers, Gardners, PA) were available ad libitum. The animal rooms were monitored for temperature, humidity and light status every 6 min. Cages were rotated on each rack once per week. The lights were on a 12 h light–12 h dark cycle with 47 lux at the bottom cages and 85 lux at the top cages. A system of dim red lighting (Phillips 15 W lamps) provided night-time lighting of <0.1 lux in the animal rooms. Temperature was maintained between 66 and 77°F (19 and 25°C), and relative humidity between 35 and 65%. The exposure room had a sound level of ~59 dB, with ~53 dB in the control room. The heating on the field generating coils at 5 G was <1°C and there was no measurable effect on temperature at the cage level. The earth’s static magnetic fields were measured in the animal rooms and varied between 0.48 and 0.54 G.

Chemicals

DMBA was purchased by the analytical chemistry laboratory, Midwest Research Institute (MRI, Kansas City, MO) from TCI America (Portland, OR). The purity determined by high-performance liquid chromatography (HPLC) was 99%, which was consistent with the purity of 98.6% indicated by the manufacturer. Sesame oil was obtained from Welch, Holme and Clark (Newark, NJ). MRI determined sesame oil identity by infrared spectrometry; the peroxide content was determined to be 0.87 ± 0.10 mEq peroxide/kg. DMBA was mixed with sesame oil to give the desired concentrations. Five replicate gavage samples of the 10 mg dose dispensed into test tubes, were received by MRI and analyzed for accuracy of dosing. Three of the five samples were selected at random and analyzed in duplicate. All analyses were within 2% of the target concentration.

Magnetic field exposure generation

The magnetic field exposure system consisted of three identical field-generating coil sets, each associated with an individual animal exposure rack in a single room. Each coil set consisted of four pairs of vertically oriented coils connected in series, which were spaced on each system. Pairs of coils were stacked one above the other; the bottom coils produced horizontal magnetic fields in one direction while the top coils produced magnetic fields opposite to those of the lower coils. This arrangement helped minimize stray fields. The wires in the coils were embedded in plastic to minimize coil vibration and hum and the coils were rotated on each rack once per week. The lights were on a 12 h light–12 h dark cycle with 47 lux at the bottom cages and 85 lux at the top cages. A system of dim red lighting (Phillips 15 W lamps) provided night-time lighting of <0.1 lux in the animal rooms. Temperature was maintained between 66 and 77°F (19 and 25°C), and relative humidity between 35 and 65%. The exposure room had a sound level of ~59 dB, with ~53 dB in the control room. The heating on the field generating coils at 5 G was <1°C and there was no measurable effect on temperature at the cage level. The earth’s static magnetic fields were measured in the animal rooms and varied between 0.48 and 0.54 G.

Exposure monitoring and control

A Gateway 2000, Model 4DX33V computer was used to drive the system and collect and store data. Multifunction synthesizers (Hewlett-Packard Model 8904A) supplied the signal to the amplifiers and were set to 50 or 60 Hz as required. The coprocessor collected data and stored it on the computer hard drive with a backup tape run each day. Emdex II field data logging units (Enertech Consultants), were used to monitor the ambient field levels for the control animals. Field levels in the exposure rooms were checked every 6 min during the 26 week study and were triggered to alarm when fields were 10% out of target field intensities. The 60 Hz stray fields did not exceed 3 mG in the 1 and 5 G 50 Hz animal exposure areas. The stray 50 Hz fields in the 1 G 60 Hz exposure areas varied from 5 to 30 mG (mean ± SD = 11.4 ± 6.4). The mean stray fields for the control animals were <1 mG. In the animal exposure areas, the target field intensity was within 10% of the measured field intensity. The long term data storage kept summaries of the exposure at 1 h intervals for each day. The exposure system fields were independently measured by Dr Martin Misakan of the National Institute of Standards and Technology (NIST) 3 days prior to study start and again after the termination of the study.

Experiment protocol

The experimental design is shown in Table I. On day 1 of the study, four groups of 130 female Sprague–Dawley rats (100 core and 30 special study) were administered; by gavage, 10 mg DMBA dissolved in 1 ml of sesame oil. Of the four groups administered DMBA, one group received no magnetic field exposure and served as a DMBA control group. An additional 130 female rats were administered 1 ml of sesame oil on day 1 of the study. These rats received no magnetic field exposure and served as a vehicle control group. Three groups of rats administered DMBA were exposed to magnetic fields at intensities/frequencies of 1 G 50 Hz, 5 G 50 Hz or 1 G 60 Hz for 18.5 h/day, 7 days/week for 26 weeks. The rats for melatonin studies were the special study animals and were killed at 4, 8 and 12 weeks of exposure/sham exposure and will be reported separately. Rats were weighed prior to study start, weekly thereafter and again at necropsy. The rats were palpated weekly; masses were located by specific mammary gland (L1–L6 and R1–R6). Two individuals each palpated half of the rats each week, alternating groups of rats. Size was determined by comparing the masses with wooden spheres of defined size. When there was a discrepancy between the previous week in number or size of the masses, then both individuals palpated the animal and resolved the discrepancy. At the time of necropsy, the clinical observations were available to the pathologist; additional masses were found at necropsy. The skin and mammary glands were removed and placed on a light box to visualize the tumors. Masses were measured in two directions and collected in the formalin. The lung, liver, kidney and all masses were fixed in formalin, stained by hematoxylin and eosin (H&E) and examined histologically. Each gross lesion was uniquely identified and followed through processing; histological diagnoses were correlated with each gross lesion identified in the mammary gland. The measurements at necropsy were used to calculate the area of the mammary gland carcinomas for each group.

Statistical methods

Comparisons of tumor incidence were made by the Poly-3 test (20–22). Comparison of tumor size and multiplicity were made by Dunnett’s test (23).

Results

Environmental conditions and field measurements

The mean room temperature for the exposure rooms was 72.2°F (22.3°C) and was within the specified range >99% of the time; the mean room temperature for the controls was

| Condition     | Start of study | End of study |
|---------------|----------------|--------------|
| 5 G 50 Hz     | 5.0 ± 0.2      | 4.6 ± 5.4    |
| 1 G 50 Hz     | 1.0 ± 0.1      | 0.9 ± 1.1    |
| 1 G 60 Hz     | 1.5 ± 0.0      | 0.9 ± 1.1    |
| DMBA control  | 0.0005 ± 0.0001| 0.0003-0.0006|

*Field intensity in G. There were two measurements in the control room but neither value exceeded 1 mG; control values are shown for the East monitor.

Table I. Experimental design

| Group         | Initiation | Promotion | Carcinogenicity study | Melatonin study¹ |
|---------------|------------|-----------|-----------------------|------------------|
| Vehicle       | None       | None      | 100                   | 30               |
| DMBA 10 mg    | 10 mg DMBA| None      | 100                   | 30               |
| 1 G 50 Hz     | 10 mg DMBA| 1 G 50 Hz | 100                   | 30               |
| 5 G 50 Hz     | 10 mg DMBA| 5 G 50 Hz | 100                   | 30               |
| 1 G 60 Hz     | 10 mg DMBA| 1 G 60 Hz | 100                   | 30               |

¹Ten animals per group were killed for analysis of serum and pineal melatonin at weeks 4, 8 and 12; these results are reported separately.
²Vehicle controls received 1 ml of sesame oil.
³Rats not exposed to magnetic fields have ambient field intensities of <1 mG.

Table II. Summary of exposure field measurements

| Condition     | Start of study | End of study |
|---------------|----------------|--------------|
| 5 G 50 Hz     | 5.0 ± 0.2      | 4.6 ± 5.4    |
| 1 G 50 Hz     | 1.0 ± 0.1      | 0.9 ± 1.1    |
| 1 G 60 Hz     | 1.5 ± 0.0      | 0.9 ± 1.1    |
| DMBA control  | 0.0005 ± 0.0001| 0.0003-0.0006|

*Field intensity in G. There were two measurements in the control room but neither value exceeded 1 mG; control values are shown for the East monitor.
Magnetic fields and mammary gland cancer in rats

Body weights and mortality

The body weights are shown in Figure 1. Rats not receiving DMBA (vehicle controls) were 5–10% heavier than rats receiving DMBA. DMBA controls were 2–7% lighter than rats receiving DMBA plus magnetic field exposure but this difference was not statistically significant. Forty-two rats (8.4%) were removed from the study; with the exception of two animals, all had mammary gland masses. The early deaths were similar among exposure groups varying from six rats in the 1 G 60 Hz group to 15 in the 1 G 50 Hz group, with 12 early deaths in the DMBA controls.

Mammary tumor palpations

The first tumors were found at 5 weeks and there was an increase in proportion of tumor-bearing rats throughout the study. Figure 2 shows that there was no difference between exposure groups and DMBA controls in the time of appearance of palpable tumors or in the proportion of tumor-bearing rats. By 13 weeks, ~50% of the rats had tumors; this reached >90% for all DMBA groups by week 26. Only two masses were found in vehicle control rats. The mean number of tumors per tumor-bearing rat at 13 weeks based on palpation was 2.3–2.9 in the exposed groups with 2.8 tumors per tumor-bearing rat in DMBA controls (Figure 3; Table III). By week 26, there was an average of six tumors per tumor-bearing rat in the DMBA controls while the exposed groups averaged 4.8–5.6 (Table III). The mean tumor size was 1.3–1.5 cm² at 13 weeks and similar at 26 weeks (Table III), probably due to the continuing appearance of new smaller masses (Figure 4). There were no differences in time of appearance, number of tumors per rat or size of tumor between DMBA controls and the various exposure groups.

Histological evaluation of mammary tumors

Necropsy and histological examination confirmed that nearly all of the masses palpated during the in-life phase of the study were either fibroadenomas (Figure 5) or adenocarcinomas (Figure 6). A few hyperplasias (one each in control, 1 G 50 Hz and 1 G 60 Hz groups) and adenomas were found (Table IV). Using the trace gross lesions identifiers, histological diagnoses were correlated with each gross lesion identified in the mam-}

72.0°F (22.2°C) and was within the specified range >99% of the time. The mean humidities of the rooms were 49 (controls) and 50% (exposed) and all rooms were within range 98% of the time. The fields in the exposure rooms are shown in Table II. The independent field measurements by NIST found values within 2% of the target values. The fields were relatively pure sine waves with a total harmonic distortion of 0.2%.

Fig. 1. Growth curves for female rats receiving either vehicle control or DMBA followed by sham or magnetic field exposure.

Fig. 2. Cumulative proportion of female rats with palpable mammary gland tumors during the 26 week DMBA initiation/magnetic field promotion study.

Table III. Evaluation of tumor size and multiplicity

| Diagnoses                  | DMBA plus magnetic field exposure group |
|----------------------------|----------------------------------------|
|                            | Control  | 1 G 50 Hz | 5 G 50 Hz | 1 G 60 Hz |
| **Palpation data (13 weeks)** |          |           |           |           |
| Tumors per tumor bearing rat | 2.8 ± 2.4| 2.4 ± 1.5 | 2.8 ± 2.5 | 2.3 ± 1.7 |
| Mean tumor size (cm²)        | 1.3 ± 0.7| 1.3 ± 0.8 | 1.5 ± 0.9 | 1.3 ± 0.8 |
| **Palpation data (26 weeks)** |          |           |           |           |
| Tumors per tumor bearing rat | 6.0 ± 4.1| 5.3 ± 3.8 | 5.6 ± 3.8 | 4.8 ± 4.0 |
| Mean tumor size (cm²)        | 1.3 ± 0.9| 1.3 ± 0.9 | 1.4 ± 1.0 | 1.4 ± 1.0 |
| **Histology data**           |          |           |           |           |
| Fibroadenoma per rat         | 3.2 ± 4.4| 3.2 ± 4.5 | 3.2 ± 3.9 | 2.8 ± 3.9 |
| Total fibroadenoma area (cm²)| 391.1    | 361.6     | 426.6     | 321.2     |
| Mean area per fibroadenoma (cm²) | 1.2    | 1.1        | 1.3        | 1.2       |
| Fibroadenoma area per rat (cm²)| 3.9 ± 6.9| 3.6 ± 7.4 | 4.3 ± 8.9 | 3.2 ± 5.9 |
| Carcinoma per rat            | 6.5 ± 4.8| 4.9 ± 4.2b| 5.5 ± 3.9 | 4.3 ± 3.9a|
| Total carcinoma area (cm²)   | 1731.6   | 1435.5    | 1815.0    | 1366.0    |
| Mean area per carcinoma (cm²)| 2.7      | 2.9        | 3.3        | 3.2       |
| Carcinoma area per rat (cm²) | 17.3 ± 16.3| 14.4 ± 16.6| 18.2 ± 18.0| 13.7 ± 18.1|

*P < 0.05 compared with controls (Dunnett’s test).
Fig. 3. Mean number of mammary gland tumors per tumor-bearing rat during the 26 week DMBA initiation/magnetic field promotion study.

Fig. 4. Mean mammary tumor size per tumor-bearing rat estimated by palpation during the in-life phase of the 26 week DMBA initiation/magnetic field promotion study.

mary gland. There were significantly fewer total carcinomas in the 1 G 50 Hz and the 1 G 60 Hz groups as compared with the DMBA controls (Figure 7; Table IV). The number of fibroadenomas was not affected by magnetic field exposure. Lung, liver and kidney were examined for the presence of neoplastic disease, especially for the presence of metastatic mammary gland carcinomas. Four DMBA control rats, four DMBA/1 G 50 Hz rats, one DMBA/5 G 50 Hz rat and four DMBA/1 G 60 Hz rats had metastatic adenocarcinomas in the lung with morphology similar to the primary mammary gland adenocarcinomas.

Evaluation of tumor size

Mammary tumor size by palpation did not differ between the exposure and DMBA control groups during the exposure period (Figure 4; Table III). The masses were also measured at necropsy and comparison with histological diagnoses allowed for calculation of the mean carcinoma area for the DMBA control and exposure groups as shown in Table III. The carcinomas were slightly larger, but not significantly increased, in exposure groups. This may have been due to the fewer number of tumors in the exposed animals; the carcinoma area per animal did not differ between the exposed and control animals. The mean fibroadenoma area was similar in all groups.

Discussion

The present study does not support the hypothesis that magnetic field exposure enhances breast cancer growth in the DMBA rat breast cancer model. In fact, there were fewer rats with tumors in the 1 G 60 Hz exposure group compared with DMBA controls. When all carcinomas were considered, the total number of carcinomas induced was lower in all magnetic field exposure groups and this was significant for the 1 G 50 Hz and 60 Hz exposure groups. There were 102 fewer carcinomas in the 5 G 50 Hz group, 155 fewer in the 1 G 50 Hz and 216 fewer in the 1 G 60 Hz exposure group. While this data is not sufficient to establish a definitive protective effect, it does suggest that we are not missing a subtle promoting effect of magnetic fields. Our data are consistent with the findings of Ekstrom et al. (24), who also observed fewer tumors in the magnetic field exposed animals compared with the DMBA controls.
Our study also had a shorter treatment regimen. The present study used a single initiating dose of 10 mg DMBA/rat while the studies of Loscher and colleagues were often an earlier onset of tumors or an increase in tumor size. There are also differences between the studies of Loscher and colleagues, the number of mammary tumors per tumor-bearing rat was not increased (11,13,25,26), and sometimes the incidence of tumors was not affected by magnetic field exposure (10,13). The positive effects reported by Loscher and colleagues were often an earlier onset of tumors or an increase in tumor size. There are also differences between the studies of Loscher and colleagues and the present study. The present study used a single initiating dose of 10 mg DMBA/rat while the studies of Loscher and colleagues used four gavage doses of 5 mg DMBA (1/week for 4 weeks) for a total dose of 20 mg rat. The study reported here used a lower DMBA dose and longer exposure time which should be more sensitive for detecting a promoting effect. Our study also had a shorter daily exposure duration (18.5 h per day, 7 days/week) while exposures in the studies of Loscher and colleagues were for 24 h/day.

Several long-term studies also failed to find an increase in mammary gland cancer in rats or mice with magnetic field exposure. In female F344 rats exposed from day 20 of gestation, through parturition, lactation and for up to 2 years, there was no evidence of increased mammary gland tumors with exposure intensities of 20 mG to 20 G (7). Male and female F344 rats exposed to 5 and 50 G for up to 2 years, had no increase in mammary gland tumors in either sex (8). Finally, in a study of 100 male and female F344 rats and B6C3F1 mice at exposure intensities of 200 mG, 2 and 10 G (both intermittent and continuous exposure) there was no evidence of increased mammary gland tumors in male or female rats or mice (9).

In summary, the DMBA initiation–promotion study reported here and other carcinogenicity studies provide little or no support that magnetic fields at 50 and 60 Hz frequency at intensities several-thousand-fold above residential exposures enhance the onset, number or growth of mammary gland tumors in the rat.

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### Table IV. Evaluation of tumor incidence

| Diagnoses       | DMBA plus magnetic field exposure group |
|-----------------|----------------------------------------|
|                 | Control* | 1 G 50 Hz | 5 G 50 Hz | 1 G 60 Hz |
| Adenoma         | 2        | 0         | 0         | 0         |
| Carcinoma       | 96       | 90        | 95        | 855       |
| Fibroadenoma    | 71       | 76        | 73        | 68        |

*Number of rats with diagnosis (100 animals examined per group).

P < 0.05 decrease compared with controls (Poly-3 test).
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