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The effects of extremely low frequency magnetic fields on mutation induction in mice

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ABSTRACT

The growing human exposure to extremely low frequency (ELF) magnetic fields has raised a considerable concern regarding their genotoxic effects. The aim of this study was to evaluate the *in vivo* effects of ELF magnetic fields irradiation on mutation induction in the germline and somatic tissues of male mice. Seven week old BALB/c × CBA/Ca F_1 hybrid males were exposed to 10, 100 or 300 μT of 50 Hz magnetic fields for 2 or 15 h. Using single-molecule PCR, the frequency of mutation at the mouse Expanded Simple Tandem Repeat (ESTR) locus *Ms6-hm* was established in sperm and blood samples of exposed and matched sham-treated males. ESTR mutation frequency was also established in sperm and blood samples taken from male mice exposed to 1 Gy of acute X-rays. The frequency of ESTR mutation in DNA samples extracted from blood of mice exposed to magnetic fields did not significantly differ from that in sham-treated controls. However, there was a marginally significant increase in mutation frequency in sperm but this was not dose-dependent. In contrast, acute exposure X-rays led to significant increases in mutation frequency in sperm and blood of exposed males. The results of our study suggest that, within the range of doses analyzed here, the *in vivo* mutagenic effects of ELF magnetic fields are likely to be minor if not negligible.

1. Introduction

In recent decades the exposure to magnetic fields has raised a considerable concern regarding their health effects [1]. The majority of the population of the developed world are currently exposed to extremely low frequency (ELF) magnetic fields produced by high voltage power lines and electrical appliances. Due to numerous epidemiological studies [2,3], IARC and WHO have classified ELF magnetic fields exposure as "possibly carcinogenic to humans" (group 2B, ref. [3]). Given that the development of cancer is a multistep process in which somatic cells acquire mutations in a specific clonal lineage [4], these results imply that magnetic fields may destabilize the genome of exposed cells. Numerous *in vitro* studies have so far failed to provide conclusive evidence for the mutagenic effects of ELF magnetic fields [5,6] and the *in vivo* genotoxic potential of MF exposure still remains poorly understood.

For technical and ethical reasons, many of the *in vivo* effects cannot be properly studied in humans, and laboratory mice therefore represent a valuable experimental model for their analysis. Over many decades the mouse model has provided the main source of experimental data used to evaluate the genetic risk of human exposure to ionizing radiation and chemical mutagens [7,8]. It should be noted that the majority of the mouse data have so far been generated using either the specific locus method (Russell 7-locus test) or the dominant lethal test. Although both tests provide important information on the mutagenicity of a number of compounds, their sensitivity is not high enough to detect mutation induction in the germline of mice following low and often intermediate doses of exposure to mutagens. To overcome this limitation, we have previously developed a highly sensitive technique for monitoring mutation induction in the germline of male mice [9,10]. This technique employs highly unstable expanded simple tandem repeat (ESTR) loci. Unstable ESTR loci consist of homogenous arrays of relatively short repeats (4–6 bp) and show a very high spontaneous mutation rates in the mouse tissues, observed as size changes in the alleles of these loci [11]. Our studies have shown that ESTR loci provide a very sensitive system for the analysis of mutation induction in the germline of male mice exposed to ionizing radiation [10,12–15], chemical mutagens [16] and anticancer drugs [17].

Abbreviations: ESTR, expanded simple tandem repeat; SM-PCR, single-molecule PCR; ELF, extremely low frequency.

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Using this system, in the present study, we have analyzed the effects of exposure to 50 Hz magnetic fields on mutation induction in the germline and somatic tissues of male mice.

2. Materials and methods

2.1. Animals

CBA/Ca and BALB/c mice were obtained from Harlan (Bicester, UK) and housed at the Public Health England, Chilton, UK. BALB/c × CBA/Ca F_1 hybrid males were bred in house for use in this study. All animal procedures were approved by the Local Ethical Review Committee and performed according to the Animals (Scientific Procedures) Act 1986 under Home Office Project Licence number PPL 30/2648. Only male mice were used in experiments, and animals were seven weeks old at time of exposure.

Groups of five mice were exposed to 50 Hz magnetic fields at 10, 100 or 300 μT for 2 or 15 h. The magnetic fields were generated using an exposure system consisting of a function generator (Hewlett Packard 3324A; HP, Bracknell, UK), a power amplifier (Kepco BOP-50-2 M; Kepco, Inc., Flushing, USA) and two pairs of Helmholtz coils. The horizontal pair (overall diameter of 54 cm) and vertical pair (overall diameter of 99 cm) were manufactured by the Clarendon Laboratory, (University of Oxford, UK) and consisted of 150 turns of 1 mm copper wire wound on a frame made from Tufnol resin. An earthed screen was wound around all coils to prevent stray electric fields. The magnetic field was measured using a 3-axis fluxgate magnetometer (MAG-03 MC, Bartington Instruments, Witney, UK) connected to a digital voltmeter (Solatron SL 6071; Solartron Metrology Limited, Leicester, UK). A personal computer running custom-made software ensured the pre-set field intensity (flux density) was maintained at ±5% of the nominal at all times; the flux density for each axis was also recorded approximately every minute. Mapping revealed that the generated field was homogenous to ±5% within the centre of the coils occupied by the mice.

Groups of five mice were given whole-body, acute irradiation of 1 Gy of X-rays (as a positive control) delivered at 0.5 Gy min^{-1} using a small animal exposure system (HS System; AGO X-rays Ltd, Aldermaston, UK). Further groups of 5 sham exposed control animals were treated identically, but without exposure to 50 Hz magnetic fields or X-rays. Testes and blood samples were collected from the exposed and sham-treated animals 12 weeks after exposure.

2.2. ESTR mutation detection by single-molecule PCR

DNA samples from sperm and blood were prepared in a laminar flow hood as previously described [13,17,18]. Approximately 5 μg of each DNA sample was digested with 25 U *Mse*I for 2 h at 37 °C; *Mse*I cleaves outside the ESTR array and distal to the PCR primer sites used for PCR amplification and was used to render genomic DNA fully soluble prior to dilution. Each digested DNA sample was diluted to approximately 10 ng μl^{-1} prior to mutation analysis.

The frequency of ESTR mutation was evaluated using a Single-Molecule PCR (SM-PCR) approach as previously described [13,17,18]. SM-PCR involves diluting bulk genomic DNA and amplifying multiple samples of DNA, each containing approximately one amplifiable ESTR molecule. The *Ms6-hm* ESTR locus consisting of pentanucleotide repeat GGGCA [19] was amplified in 10 μl reactions using 0.4 μM flanking primers HM1.1F (5′-AGA GTT TCT AGT TGC TGT GA-3′) and HM1.1R (5′-GAG AGT CAG TTC TAA GGC AT-3′). Amplification was performed using 0.035 U μl^{-1} of the Expand High Fidelity PCR System (Roche, Mannheim, Germany), 1 M Betaine and 200 μM each deoxynucleotide triphosphate. Amplification was carried out at 96 °C (20 s), 58 °C (30 s), 68 °C (3 min) for 30 cycles on a PTC-225 DNA Engine Tetrad (MJ Research, Waltham, Mass). To increase the robustness of the estimates of individual ESTR mutation frequencies, on average 135 amplifiable molecules were analyzed for each tissue for each mouse.

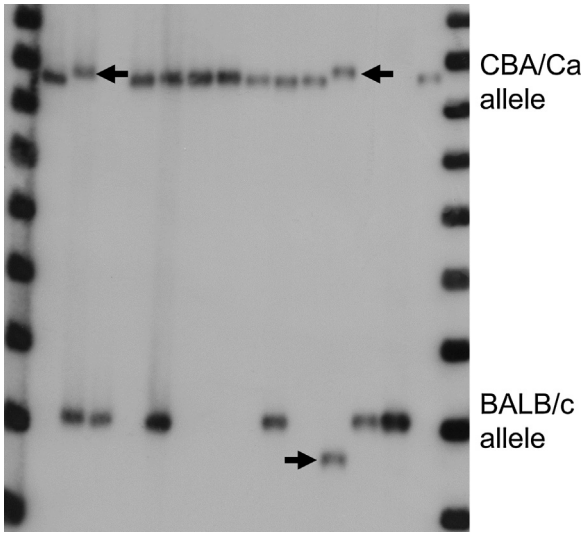

Fig. 1. Mutation detection at the *Ms6-hm* ESTR locus by SM-PCR. Mutants are indicated by arrows.

PCR products were resolved on a 40-cm long agarose gel and detected by Southern blot hybridization to ^{32}P-dCTP labelled probe as previously described [10]. The number of amplifiable molecules, ESTR mutation frequencies and their standard errors were estimated using a modified approach proposed by Chakraborty et al. [20]. The frequency of ESTR mutation, μ in each tissue was estimated by dividing the number of mutants, m by the total number of amplifiable DNA molecules, λ. The standard error of mutation frequency, $se\mu$ was estimated as:

$$se\mu = \mu\sqrt{\left(\frac{se\lambda}{\lambda}\right)^2 + \frac{1}{m}}$$

where $se\lambda$ is the standard error of the number of amplifiable DNA molecules. The Student's test was used to compare the frequency of ESTR mutation in exposed and sham-treated mice.

3. Results

3.1. Experimental design and ESTR mutation detection

Mutations were scored at both the smaller BALB/c-derived allele (~2.5 kb) and the larger (~3.5 kb) CBA/Ca allele (Fig. 1). As in our previous studies, only bands showing a shift of at least 1 mm relative to the progenitor allele were scored as mutants. By sacrificing animals 12 weeks after treatment it was ensured that the sperm assessed were derived from exposed A_s spermatogonia [21]. According to the results of our previous studies, exposure to ionizing radiation [10,22] and chemical mutagens [16] results in highly significant increases in ESTR mutation rate in males exposed at this particular stage of mouse spermatogenesis.

The magnetic field strengths of 10, 100 and 300 μT were chosen to reflect the ICNIRP guidelines for limiting residential exposure to 50 Hz magnetic fields [23]. Male mice exposed to 1 Gy of acute X-rays were used as a positive control. Given the results of our previous studies, such an exposure significantly increases the frequency of ESTR mutation in DNA samples taken from sperm of irradiated mice [13,15].