Mutagenic activity of ELF magnetic fields

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ABSTRACT

Possible carcinogenic and/or mutagenic activity of extremely low frequency magnetic fields was estimated using somatic mutation and recombination test system of *Drosophila melanogaster*. A DNA repair defective mutation *mei-41*^{D5} was introduced into the conventional *mwh/flr* test system to enhance mutant spot frequency.

w mei-41^{D5}*/FM6; flr/TM6* females were crossed to *w mei-41*^{D5}*/Y; mwh jv; spa*^{pol} males. The F_1 third instar larvae were exposed to a 50Hz, 20mT AC magnetic field for 24h. After moulting from pupal cases, their wings were examined under a bright field microscope to detect hair spots with *mwh* or *flr* mutant morphology.

The exposure caused a statistically significant enhancement in somatic recombination spot frequency compared to the unexposed control. Mutant spots arising due to chromosomal non-disjunction or terminal deletion also increased but the frequency of spots resulting from point mutation was not altered. The enhancement in the recombination spot frequency was suppressed to the control level when a culture medium without electrolytes was used during exposure. When larvae were exposed to a magnetic field in an annular dish, flies from the outer ring showed more mutant spots than those from the inner dish. These results suggest that the detected mutagenic activity was that of the induced eddy current, rather than that of the magnetic field itself.

Introduction

Recently, possible biological effects of extremely low frequency (ELF) electromagnetic fields have been extensively studied (1). Most of the recent studies focus on the adverse effects to human health, especially on the cancer causing effect of magnetic fields around power-lines or electrical appliances. Flux density used in these experimental studies were close to that of the magnetic field we are actually exposed to. The results were often ambiguous and contradictory to each other, possibly due to weakness of flux density.

Miyakoshi et al. (2), on the other hand, analyzed mutagenic activity of very strong ELF magnetic fields up to 400mT. They reported that exposure to the field caused mutation in cultured human MeWo cells, and that the induced current, rather than the magnetic field itself seemed to be mutagenic.

We have reported that carcinogenic and/or mutagenic activity of static magnetic fields could be estimated *in vivo* using the somatic mutation and recombination test (SMART) system of *Drosophila melanogaster* such as developmental lethality test (3) or wing spot test (4). Here we applied the wing spot test system to an ELF magnetic field and could obtain data that support the conclusion of Miyakoshi et al. (2).

Materials and Methods

A DNA repair defective mutation *mei-41*^{D5} (5) was introduced into the conventional *mwh/flr* test system (6) to enhance mutant spot frequency. Virgin females of *w mei-41*^{D5}*/FM6; flr/TM6* were crossed to *w mei-41*^{D5}*/Y; mwh jv; spa*^{pol} males. For details about genes and balancer chromosomes, see Lindsley and Zimm (7). Three to four days old parents were allowed to lay eggs for 24h. After parents were removed, the eggs were incubated for 48h under normal culture condition. Then culture vials were placed in the exposure bore of a Helmholtz coil (ISM-12K14-2, Tokyo Denshi Ltd.) and the larvae were exposed to a 50Hz, 20mT horizontal AC magnetic field for 24h. Thereafter, they were reared under normal culture condition again.

Among eight kinds of F_1 progeny, *w mei-41*^{D5}*/FM6; flr/mwh jv; spa*^{pol}/+ tester females and balancer heterozygous *w mei-41*^{D5}*/FM6; mwh jv/TM6; spa*^{pol}/+ females were discriminated by the external marker mutation and were used for further analyses. After moulting from pupal cases, their wings were excised, mounted on a glass slide with Faure's solution and were examined under a bright field microscope (400×) to detect hair spots with *mwh* or *flr* mutant morphology. Large mutant spots with 3 or more mutant hairs and small spots with 1 or 2 mutant hairs were counted separately. Conventional culture medium (corn-meal, sugar, agar and yeast with 0.8% propionic acid) and vials (30mmϕ×100mmH) were used except when stated otherwise. All experiments were carried out in constant temperature rooms at 24±0.5°C.

Results and Discussion

[Mutant spots were induced by exposure to an ELF magnetic field]

Four types of mutant spot appeared at a low frequency even in the control experiment. They were small single, *flr* large single, *mwh* large single and twin spots (*flr* and *mwh* spots appearing side by side). Small spot per wing was about 0.5 in *w mei-41^{D5}/FM6; flr/mwh jv; spa^{pol}/+* tester females, while large and twin spot was 0.06 and 0.04, respectively (Table 1). Chromosomal non-disjunction and terminal deletion are considered to cause small spots. Among large single spots, *flr* spots are inferred to result from point mutation while *mwh* spots result from either point mutation or somatic recombination between *flr* and *mwh* loci. Twin spots, on the other hand, result solely from somatic recombination between *flr* and centromere. Our results indicates that the frequency of somatic recombination is less than that of non-disjunction and terminal deletion, and that point mutation occurs even less frequently in the control.

In *w mei-41^{D5}/FM6; mwh jv/TM6; spa^{pol}/+* females, somatic recombination in the third chromosome is totally suppressed by multiple reversions in TM6 chromosome, and hence, twin spots did not appear (Table 1). Large spots were observed in a lower frequency as they result from only point mutation. Therefore, (large *mwh* spot + twin spot) frequency in the tester females minus large *mwh* spot frequency in the balancer heterozygote makes net somatic recombination frequency. It was 0.07 (=0.06+0.04–0.03) in the control.

When flies were exposed to a 50Hz, 20mT ELF magnetic field for 24h, net somatic recombination frequency increased to 0.17 (Table 1). Small spots also increased, while change in point mutation frequency was not significant. These results suggest that exposure to an ELF magnetic field caused mutation at chromosomal level rather than at DNA level. Electric conductivity of the culture medium was 0.4S/m. The eddy current induced in the culture medium in which larvae were reared was calculated to be about 18mA./m^2 Detectable temperature increase (>0.1°C) during exposure was not observed. Therefore, increase in the mutant spot frequency seems to result from magnetic field exposure rather than an artifact.

Table 1. Wing spots induced by exposure to a 50Hz, 20mT ELF magnetic field

	# of wings observed	small spot per wing	large *flr* spot per wing	large *mwh* spot per wing	twin spot per wing
control					
mwh/flr[a)]	363	0.50	0.003	0.06	0.04
mwh/TM6[b)]	286	0.49	0.0	0.03	0.0
			somatic recombination frequency[c)]=0.07		
exposed					
mwh/flr[a)]	380	0.65*	0.005	0.16	0.04
mwh/TM6[b)]	213	0.56*	0.0	0.03	0.0
			somatic recombination frequency[c)]=0.17*		

a)*w mei-41^{D5}/FM6; flr/mwh jv; spa^{pol}/+* tester females

b)*w mei-41^{D5}/FM6; mwh jv/TM6; spa^{pol}/+* females

c)somatic recombination frequency=(large *mwh* spot/wing + twin spot/wing) in *mwh/flr* – large *mwh* spot/wing in *mwh/TM6*

*:significant at 1% level

[Influence of electrolytes]

As exposure to an ELF magnetic field caused somatic recombination and other chromosomal aberration, the effects of eddy current should be suspected. The larvae live not on the surface of the culture medium but crawl in it, therefore eddy current induced in the medium should be considered rather than the current induced in individual larvae. We prepared culture medium containing no electrolytes and performed the same exposure using this medium (50Hz, 20mT×24h). The medium was consisted of 1% glucose, 0.8% agar and Milli-Q water, and its electric conductivity was 0.01S/m. Spot frequency in *w mei-41^{D5}/FM6; flr/mwh jv; spa^{pol}/+* tester females was examined . Difference in spot frequency between exposed and control groups was statistically non-significant (Table 2).

Table 2. Influence of electrolytes on the wing spot frequency in tester females[a)]

	# of wings observed	small spot per wing	large *flr* spot per wing	large *mwh* spot per wing	twin spot per wing
Sugar+Agar medium, σ=0.01S/m					
control	146	0.49	0.0	0.04	0.02
exposed	197	0.54	0.0	0.06	0.03
Sugar+Agar medium+0.5%NaCl, σ=0.8S/m					
control	143	0.52	0.0	0.04	0.02
exposed	178	0.78	0.01	0.17	0.04

a) spot frequency in *w mei-41^{D5}/FM6; flr/mwh jv; spa^{pol}*/+ tester females was examined

When 0.5% NaCl was added to this medium, electric conductivity rose to 0.8S/m, and spot frequency in exposed group became significantly larger than that in control group (Table 2) As the control value was not influenced by salt, the electric, rather than chemical, character of media seemed to be responsible for the difference. Therefore, involvement of eddy current induced in the medium was strongly suggested.

[Exposure using an annular dish]

To confirm the hypothesis that the eddy current is the cause of increase in the spot frequency, an exposure using an annular dish was performed (Fig. 1). Larvae were exposed to a 50Hz, 20mT magnetic field for 24h, and spot frequency in *w mei-41^{D5}/FM6; flr/mwh jv; spa^{pol}*/+ tester females was examined.

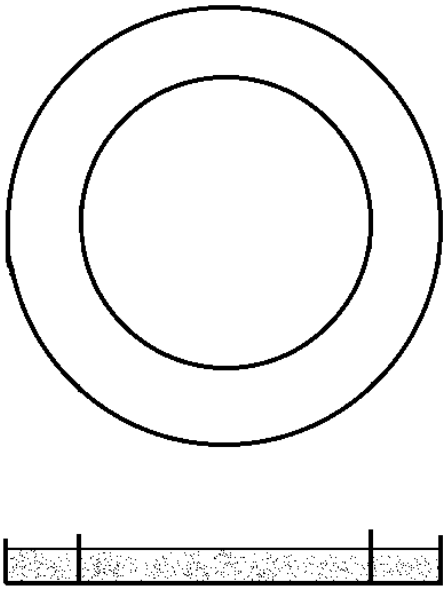

Fig. 1 A ground plan (upper) and an elevation (lower) of annular dish. The outer and inner rings are filled with culture medium and electrically insulated by the wall of the inner ring.

When a dish is placed perpendicular to the magnetic flux, the eddy current induced in the medium should be proportional to the radius of dish. We could expect that flies exposed in the outer ring would have more mutant spots than those reared in the inner ring. Table 3 shows that this was actually observed, and eddy current was inferred to be involved. These results suggest that the induced eddy current, rather than the magnetic field itself, caused aberration at chromosomal level.

Table 3. Wing spot frequency of flies exposed to an ELF magnetic field in an annular dish

	# of wings observed	small spot per wing	large *flr* spot per wing	large *mwh* spot per wing	twin spot per wing
Inner ring	186	0.56	0.02	0.08	0.02
Outer ring	147	0.74	0.02	0.11	0.03

Spot frequency in *w mei-41^{D5}/FM6; flr/mwh jv; spa^{pol}/+* tester females was examined

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